EVALUATION OF POLYCYSTIN-1 EXPRESSION IN DENTIGEROUS CYST AND ASYMPTOMATIC DENTAL FOLLICLE

Dissertation submitted to

THE TAMILNADU Dr. M.G.R.MEDICAL UNIVERSITY

In partial fulfillment for the Degree of

MASTER OF DENTAL SURGERY

BRANCH VI

ORAL PATHOLOGY AND MICROBIOLOGY

APIRL 2013

CERTIFICATE

This is to certify that this dissertation titled "**EVALUATION OF POLYCYSTIN-1 EXPRESSION IN DENTIGEROUS CYST AND ASYMPTOMATIC DENTAL FOLLICLE**" is a bonafide dissertation performed by **JAISHLAL.M.S** under our guidance during his postgraduate period 2010-2013.

This dissertation is submitted to **THE TAMILNADU Dr.M.G.R. MEDICAL UNIVERSITY,** in partial fulfillment for the degree of **MASTER OF DENTAL SURGERY** in **ORAL PATHOLOGY AND MICROBIOLOGY, BRANCH VI**. It has not been submitted (partial or full) for the award of any other degree or diploma.

Guided By

Dr. K. Ranganathan, M.D.S., MS (Ohio), PhD
Professor & HOD,
Dept. of Oral & Maxillofacial Pathology
Ragas Dental College & Hospital,
Chennai.

Dr. K. Ranganathan, MDS, MS(Ohio), Ph.D,
Professor and Head,
Department of Oral and Maxillofacial Pathology
Ragas Dental College and Hospital, Chennai

Dr. T. Rooban, M.D.S.,
Professor,
Dept. of Oral & Maxillofacial Pathology
Ragas Dental College & Hospital,
Chennai.

Dr. T. Rooban, MDS
Professor
Ragas Dental College and Hospital, Chennai

Dr. S. Ramachandran, M.D.S.,
Principal,
Ragas Dental College & Hospital
Chennai.

PRINCIPAL
RAGAS DENTAL COLLEGE & HOSPITAL
CHENNAI

Acknowledgement

My thesis could not have been completed without the help of many people to whom I am indebted. My heartfelt gratitude to my post graduate teacher and mentor ***Dr. K. Ranganathan*** *MDS, MS (Ohio), PhD, Professor and Head, Department of Oral and Maxillofacial Pathology, Ragas Dental College and Hospital, for his constant support and encouragement and for guiding me throughout my dissertation.*

I would like to express my real sense of respect and thanks to my ***Guide Dr. T. Rooban M.D.S.,*** *Professor, Department of Oral and Maxillofacial Pathology, Ragas Dental College and Hospital, for his guidance, patience, immense knowledge, motivation and valuable criticism extended to me during the period of my study. The timely help and encouragement rendered by him has been enormously helpful throughout the period of my postgraduate study.*

My most sincere thanks to ***Dr. Umadevi K Rao*** *and* ***Dr. Elizabeth Joshua*** *Professors, Department of Oral and Maxillofacial Pathology, Ragas Dental College and Hospital, for their guidance and encouragement throughout my study.*

I am thankful to the Principal ***Dr. S. Ramachandran*** *and Chairman Professor* ***Mr. A. Kanagaraj****, Ragas Dental College and Hospital, for their permission to use the facilities of the esteemed institution.*

I also thank ***Dr. K.M. Vidya, Dr. N. Lavanya, Dr. C. Lavanya,*** *Department of Oral and Maxillofacial Pathology, Ragas Dental College and Hospital, for their constant motivation and support throughout my study.*

I am deeply indebted to the Directors, Doctors and Paramedical staff members of ***MADURAI KIDNEY CENTRE*** *for granting me permission and helping me to oral screen the patients with renal disease for my study.*

I am very grateful to our Research Assistant ***Mrs. Kavitha Wilson,*** *and Lab Technician* ***Mr. Rajan****, Department of Oral and Maxillofacial Pathology, Ragas Dental College and Hospital, for their help in the completion of my study.*

I deeply acknowledge the help of my batchmates ***Dr. Aiswarya Lekshmy S.U, Dr. Nithya S, Dr. Shanmuga Priya P, Dr. Sudharsan R and Dr. Vaishnavi S*** *and my* ***seniors, juniors*** *for their support and encouragement.*

I specially thank ***my parents, my family and friends*** *for their constant love, understanding, support and prayers throughout my curriculum.*

I sincerely thank my ***Patients*** *without whom this study would not have been complete.*

Above all I thank GOD almighty for the grace and blessings endowed upon me.

ABSTRACT

Background: Primary cilium has been hypothesized to play a role in the pathogenesis of dentigerous cyst. The role of primary cilium associated protein, Polycystin-1, that has been associated/ implicated in autosomal dominant polycystic kidney diseases has not been studied in dentigerous cysts.

Aim and Objective: To study and compare the expression of Polycystin-1 in dental follicular tissue and dentigerous cyst and to identify the prevalence of dentigerous cyst in chronic renal disease patients.

Materials and Methods: Formalin-fixed, paraffin-embedded tissue sections of Dental follicles (n=31) and Dentigerous cyst (n=15) which were confirmed clinically, radiographically and histopathologically were included in this study. The sections were stained with antibodies against Polycystin-1 and staining intensity was graded as nil (0), mild (+) , moderate (++) and intense (+++) . Oral screening was also done for 100 patients with chronic renal diseases.

Results: Polycystin-1 expression between dentigerous cyst and dental follicles were statistically significant ($P < 0.05$). The intensity of staining varied between the epithelial cells of dentigerous cyst and dental follicles. It was observed that no clinical evidence of dentigerous cyst was present in 100 chronic renal disease patients.

Conclusion: The significant difference in the expression of Polycystin-1 between dentigerous cyst and dental follicle suggests that Polycystin-1 can have a role in the transformation of dental follicle to dentigerous cyst.

Keywords: Polycystin-1, Dentigerous cyst, Dental follicle, Impacted tooth, Reduced enamel epithelium.

CONTENTS

S. NO.	INDEX	PAGE NO.
1.	INTRODUCTION	1
2.	AIMS AND OBJECTIVES	3
3.	MATERIALS AND METHODS	4
4.	REVIEW OF LITERATURE	16
5.	RESULTS	54
6.	DISCUSSION	62
7.	SUMMARY AND CONCLUSION	70
8.	BIBLIOGRAPHY	71
9.	ANNEXURES	-

Dentigerous cyst (DC) is the second most common type of developmental odontogenic cyst. DC is widely believed to originate by fluid accumulation between REE and tooth, and/or proliferation of the associated odontogenic epithelium. Differentiating early DC from an enlarged or dilated normal pericoronal space can be difficult. [1-4] In radiographs, the thin radiolucent line that surrounds the impacted or erupting tooth represents the position of post-functional, REE, fibrous tissue, and odontogenic rests. This tissue is referred to as the Dental follicular tissue in this dissertation. It has been referred in literature as dental follicle and shall not be confused with developmental odontogenic tissue with the same name.

Clinical, radiological and histopathological features are necessary for the differentiation of early DC from dilated follicle. [4] This differentiation is necessary as their behaviors are different. DC has potential to transform in to ameloblastoma, mucoepidermoid carcinoma or squamous cell carcinoma.[1,4] There are several hypothesis for the aetiopathogenesis of DC. One of the hypotheses gaining interest is the role of primary cilium, its associated proteins such as Polycystin-1 and calcium transport channels.[5] Polycystin-1, a primary cilium associated protein, is the product of gene PKD1 and in addition to its suspected role in aetiopathogenesis of DC has been associated with Autosomal Dominant Polycystic Kidney Diseases (ADPKD). ADPKD is an inheritable genetic condition where in a gene, PKD1 encoding for Polycystin1 (PC1) mapping to chromosome 16p13.3 is mutated in 85% of instances.[5]

This study attempts to identify and correlate the expression of the PC1 in DC and dilated follicles.

Given the role of PC-1 in renal diseases, we also wanted to ascertain if patients with renal diseases and ADPKD have an increase prevalence of dentigerous cyst.[6-9]

AIM

1. To study the expression of Polycystin-1 in follicular tissue of unerupted teeth.
2. To study the expression of Polycystin-1 in dentigerous cyst.
3. To compare Polycystin-1 expression in follicular tissue and dentigerous cyst.
4. To identify the prevalence of dentigerous cyst in chronic renal disease patients.

OBJECTIVES

- To identify Polycystin-1 in follicle and dentigerous cyst using antibody to Polycystin-1 (7e12 epitope of end terminal region of human Polycystin-1) by immunohistochemical techniques in archival, paraffin embedded sections.
- To correlate the prevalence of dentigerous cyst in chronic renal failure patients.

ALTERNATE HYPOTHESIS

- Increased Polycystin-1 expression is observed in dentigerous cyst.
- Dentigerous cyst is more prevalent in chronic renal failure patients.

STUDY SETTING:

The study was conducted in Department of Oral and Maxillofacial Pathology, Ragas Dental College and Hospital, Chennai, archival formalin fixed paraffin embedded Follicle and Dentigerous cyst. The expression of Polycystin-1 in Follicles and Dentigerous cyst was studied by Immunohistochemistry (IHC).

Ethical clearance for the study was obtained from the Institutional Review Board (IRB) of Ragas Dental College (Annexure-I) and protocol submitted to IRB (Annexure- II). When the tissues were collected prospectively, consent was obtained from all patients.

Sample Size:

- A total of 41 follicles were obtained. Ten samples were excluded due to the absence of epithelium. Thirty one samples were taken for evaluation.
- Clinically, radiologically and histopathologically confirmed 15 cases of Dentigerous cyst were included.
- Oral health screenings of 100 chronic renal disease patients were done.

Sample size calculation: To study the expression of Polycystin-1 in dentigerous cyst and follicles, for a power of 80%, at 5% significant level, 15 dentigerous cyst and 31 follicles were studied.

Criteria for Dental follicles (DF):

Dental follicle was obtained from 40 patients reporting to Ragas Dental College and Hospital for removal of impacted teeth. (partial, complete) third molars (maxillary/mandible) and canines. After the tooth was removed, the soft tissue associated with crown of impacted tooth was enucleated from the attachment and procured for histopathological analysis. Tissues thus obtained were then fixed in 10% buffered formalin and then processed and embedded in paraffin.

Follicle was categorized based on the following features.

- Inclusion criteria consisted of cases with impacted third molars/ canine that was completely/partially surrounded by bone. The longest distance from the unerupted crown to the adjacent opaque bone was considered as the width of the pericoronal space. The present study was performed to microscopically evaluate the pericoronal tissues of impacted tooth with radiolucency less than 2 millimeters. Several studies have sited radiographic width greater than 4 millimeters to be associated with the odontogenic pathology. To ensure to collect only non-pathological follicular tissues, only impacted teeth with radiolucent pericoronal width less than 2 mm were considered for the study.

- Radiographic impaction type's distoangular/ mesioangular were also recorded for all cases. The following microscopic parameters were recorded for each case: Presence of epithelium and its type [stratified squamous (non-keratnized/para/ortho), cuboidal and columnar], connective tissue pattern (fibrous, myxomatous or both), presence of odontogenic epithelial rests and

calcifications, chronic non-specific inflammation, and existence of other cystic or neoplastic lesions. (Annexure-III)

Criteria for Dentigerous cyst:

- A Dentigerous cyst is an epithelium lined pathlogical cavity enclosing the crown of the unerupted tooth by expansion of follicle and is attached to the neck of the tooth. Epithelium could be keratinized/non-keratinized/ciliated/columnar or REE type.

- Clinically, radiologically and histopathologically confirmed 15 cases (n = 15) of DC with all demographic and clinical details were retrieved from archives and used for the study. Demographic and histopathological features including age, gender, clinical duration, inflammation, site, radiological and histopathological features were noted down.

- Five micron thick sections were cut and used for routine hematoxylin and eosin (H&E) from DC and DF tissues.

- H & E staining and immunohistochemical (IHC) staining was performed as below

HEMATOXYLIN & EOSIN STAINING

REAGENTS

- ✓ Harris hematoxylin
- ✓ 1% acid alcohol
- ✓ Eosin

PROCEDURE

- ✓ The slides were dewaxed in xylene and hydrated through graded alcohol to water.
- ✓ The sections on the slides were flooded with Harris hematoxylin for 5 minutes.
- ✓ The slides were washed in running tap water for 5 minutes.
- ✓ The slides were differentiated in 1% acid alcohol for 5 minutes.
- ✓ The slides were washed well in running tap water for 5 minutes.
- ✓ The tissue sections on the slides were then stained in eosin for 30 seconds.
- ✓ The slides were washed in running tap water for 1 minute.
- ✓ The slides were then dehydrated through alcohol, cleared, mounted and viewed under light microscope (LM).

IMMUNOHISTOCHEMISTRY (IHC)

ARMAMENTARIUM

- ✓ Microtome
- ✓ Autoclave
- ✓ Hot air oven
- ✓ Slide warmer
- ✓ Coupling jars
- ✓ Measuring jar
- ✓ Weighing machine

- ✓ APES coated slides
- ✓ Slide carrier
- ✓ Aluminum foil
- ✓ Micro-pipettes
- ✓ Toothed forceps
- ✓ Electronic timer
- ✓ Beakers
- ✓ Rectangular steel tray with glass rods
- ✓ Sterile gauze
- ✓ Cover-slips
- ✓ Light microscope

REAGENTS USED

1. Conc. Hydrochloric acid, Analytical Grade
2. Laxbro solution.
3. APES (3 amino propyl tri ethoxysilane).
4. Acetone.
5. Citrate buffer.
6. Tris Buffer.
7. 3% H_2O_2
8. Deionized distilled water.
9. Absolute alcohol.
10. Xylene.

ANTIBODIES USED

Primary antibody– Anti- POLYCYSTIN-1 Antibody (7e12) (Abcam, Cambridge, MA,USA) Mouse monoclonal; Developed against bacterially expressed fusion protein from the N-terminal leucine rich repeats (LRR) domain of human Polycystin-1. The epitope [7e12] was produced to the flank-leucine rich repeat-flank region. Concentrated; recommended dilution as per manufacturer instruction.

Positive control: This antibody gave a positive signal when used to probe a normal human liver tissue section.

Secondary antibody – The Universal, HRP Detection System based on proprietary CRF^{TM} Technology (AbCAM, USA) is a combination of traditional Biotin-Streptavidin system contains Peroxide Block for image analysis, Super block, CRF^{TM} Anti-Polyvalent Horse Radish Peroxidase, Di-Amino Benzidine (DAB) Chromogen Concentrate and DAB Substrate which is effective with antibodies of mouse, rat, rabbit and guinea pig.

IHC PROCEDURE

PRETREATMENT OF THE SLIDES

- ✓ The slides were first washed in tap water for few minutes.
- ✓ The slides were then soaked in detergent solution for 1 hour.
- ✓ After 1 hour, each slide was brushed individually using the detergent solution and were transferred to distilled water.
- ✓ The slides were washed in two changes of distilled water.

- ✓ The slides were washed in autoclaved distilled water.
- ✓ The slides were immersed in 1 N Hydrochloric acid (Hcl) (100 ml Hcl in 900 ml distilled water) overnight.
- ✓ The following day, slides were taken out of acid and washed in two changes of autoclaved distilled water.
- ✓ All the slides were then transferred to slide trays, wrapped in aluminium foil and baked in hot air oven for 4 hours at 180 degrees centigrade.

APES (3 Amino propyl tri ethoxysilane) coating

Slides first dipped in couplin jar containing acetone for 2 minutes

↓

Dipped in APES for 5 minutes

↓

Dipped in two changes of distilled water for 2 minutes each

↓

Slides left to dry

PREPARATION OF PARAFFIN SECTIONS

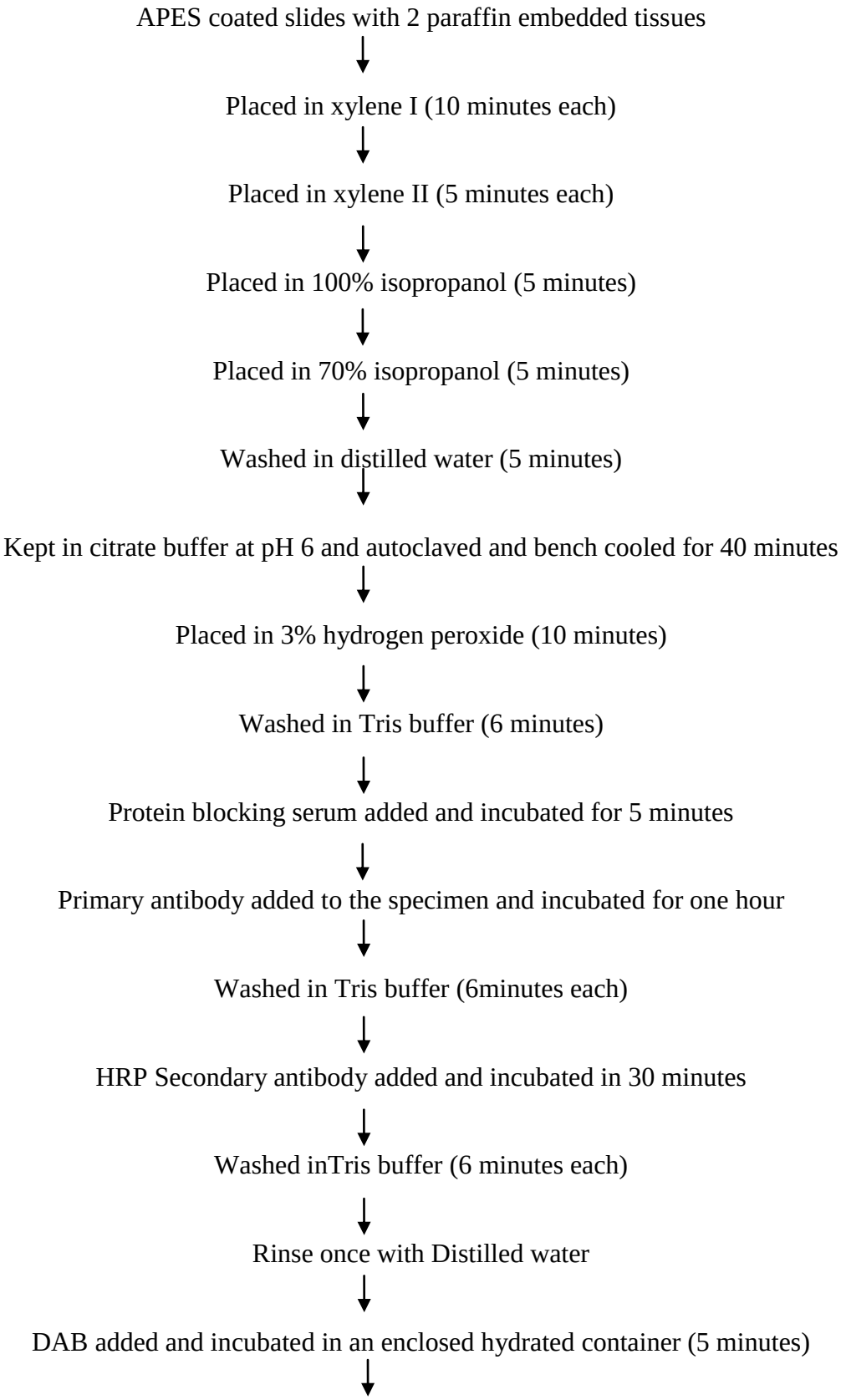
After the slides were dry, tissue section of 5 micron thickness were made in a rotary manual microtome. The ribbons of tissue section were transferred onto the APES coated slide from the tissue float bath such that two tissue bits come on to the slide with a gap in between. One of the tissue sections was labeled positive (P) and the other negative (N).

PROCEDURE

The slides with tissue sections were treated with three changes of xylene to remove paraffin wax. They were put in descending grades of alcohol and then

rehydrated with water. The slides were then transferred to citrate buffer and autoclaved for antigen retrieval at 15 pounds pressure for 30 minutes. Slides were then treated with 3% hydrogen peroxide for 10 minutes to quench endogenous peroxidase activity of cells that would otherwise result in non – specific staining. The slides were dipped in 3 changes of Tris buffer for 6 minutes. Circles were drawn around the tissues, so that the antibodies added later on do not spread and are restricted to the circle. The tissues were incubated in protein blocking serum (Super block) for 5 min in an enclosed hydrated container. Then the slides were wiped carefully without touching the tissue section to remove excess of blocking serum. The primary antibody, Polycystin-1, diluted as per manufacturer instruction was added to positive tissue on the slide and then to the negative tissue Tris buffer was added. The slides were incubated for 1 hour. Then the slides were wiped carefully without touching the tissue section to remove excess of antibody and washed with three changes of Tris buffer for 6 minutes. Then secondary antibody, CRF^{TM} anti-polyvalent HRP polymer was added on both the sections and the slides were incubated for 30 minutes. Later slides were washed in three changes of Tris buffer for 6 minutes in each. The slides were wiped carefully without touching the tissue section to remove excess Tris buffer. The sections were rinsed in distilled water for 2 minutes. Then the slides were wiped carefully without touching the tissue section to remove excess distilled water. Then a drop of DAB was added to the sections for 5 minutes. Slides were then washed in distilled water to remove excess chromogen and counter stained with hematoxylin. Then the slides were transferred to 70% alcohol, 100% alcohol and one change of xylene. The tissue sections were mounted with DPX. The slides were then observed under the microscope. Throughout the procedure care was taken not to dry the tissues.

IHC PROCEDURE FLOW CHART



Rinse once with Distilled water

↓

Stained with hematoxylin(20 seconds)

↓

Washed in tap water

↓

Slides were mounted using DPX

↓

Slides were observed under the Light Microscope and graded

The follicle and dentigerous cyst slides where stained with antibodies against Polycystin-1 in batches of 9 with each batch having 1 liver slide as a positive control. The presence of stains were measured by grading the intensity of staining as nil, Mild, Moderate and Intense.

Reduced enamel epithelium (that makes the dentigerous cyst/follicle lining) is made up of inner enamel epithelium and outer enamel epithelium, which often constitutes the lining of dentigerous cyst, two epithelial cell layers were considered as basal cells and all other layers were considered as suprabasal cells.

A minimum of 100 cells in each section (basal and if suprabasal layers were present, separately) in a total of 5 foci were counted in 10 X and 40 X magnifications. In cases that lacked 100 cells, all cells available in the section (basal/suprabasal) was counted. A tissue was assumed to be PC-1 positive when 80% of counted epithelial cells were stained with PC-1. The positivity of PC-1 staining in basal and suprabasal layers, cellular localization (membrane/nuclear/cytoplasmic) was recorded. The overall PC-1 staining was graded as nil (0), mild (+), moderate (++) and Intense (+++) based on the percentage of cells that had the degree of staining. As there were only very few

epithelial cells present, especially in follicular tissues and basal cells, no intensity evaluation was performed and compared. In DC, the suprabasal cells often were separated as per reports in literature compounding the problem of evaluating the intensity and comparing it.

Oral health screening of 100 chronic renal disease patients was performed. Screening of renal disease patients was performed at Madurai Kidney Centre, Madurai. Following written Permission from hospital authorities and a verbal consent from all patients, oral examination was done (Annexure-IV). Demographic details as well as past medical history of the renal disease patients were recorded in a predetermined format. A thorough oral examination was done and findings were recorded in prestructured case sheets (Annexure-V). No radiological examination was performed.

Definitions of Acute Renal failure, Chronic Renal failure, End stage renal disorders and oral lesions identified are given in Annexure-VI.

ARMAMENTARIUM

For patient examination

- Gloves and mouth masks
- Sterile mouth mirror and straight probe

ORAL EXAMINATION

Patient was asked to open the mouth widely and oral screening done by using mouth mirror and straight probe, the data was immediately entered in the prestructured

case sheets. Diagnostic criteria given in Annexure V were employed for identifying oral lesions.

All patients were examined by a single examiner and well calibrated for definition in Annexure VI.

Oral lesions identified were written in the case sheets and referred with opinion for subsequent management to concerned hospital authorities.

Statistics:

Data collected were entered and analyzed using Statistical Package for Social Science, 17.0 (SPSS, IBM, IL, USA). Descriptive statistics were presented for all variables. One way ANOVA with 95% confidence interval (CI) was used to compare the mean age, mean duration of inflammation. Chi square test were employed to identify the difference between predictor (gender, impaction status, inflammation) and outcome variables (histological features and PC-1 staining features. A P value of less than or equal to 0.05 was taken as statistically significant.

DENTAL FOLLICLE

An impacted tooth is one of the most prevalent problems in young adults examined in the dental office. Dental follicle associated with these unerupted teeth is the remnant of tissue that has participated in odontogenesis and remained circumjacent to the crown of a tooth. Despite its role in eruption physiology, studies have reported that long standing DF may undergo cystic degeneration and/or neoplastic transformation.[12] The thin radiolucent line that surrounds the erupting teeth is the post-functional, reduced enamel epithelium and the remnant DF and often referred as peri-follicular tissue.[2] DF is composed of fibrous connective tissue and frequently contains epithelial residues of odontogenesis, which could be the potential starting point of pathology.[2] DF are comprised of fibrous tissue, nests of odontogenic epithelium and the reduced enamel epithelium. DF often remain adjacent to the crown of impacted teeth, and occasionally lead to the development of cysts or tumors, such as DC, keratocyst or ameloblastoma, that arise from odontogenic epithelial rests.[3]

DCs were believed to originate initially by proliferation and cystic transformation of islands of epithelium in the connective tissue wall of the DF and this transformed epithelium then unites with the lining epithelium and forms a solitary cyst around the crown of the tooth.[4] Certain other authors believe that DCs originate by the fluid accumulation (owing to various factors) between the enamel of the tooth and the reduced enamel epithelium. The origin of DC remains controversial.[4]

RADIOGRAPHICAL FEATURES:

Radiographic interpretations of non-symptomatic impacted third molars without any other obvious clinical diagnostic features are assumed to be normal. Previous literature suggests that pericoronal radiolucency of <2.5mm in width is most oftennonpathologic.[13] It is reported that third molars that are overdue for normal eruption but remain impacted may subsequently develop pathologies, over a period of time. The decision to remove an impacted tooth is less challenging when signs and symptoms of pathosis are present, but it is more demanding when the patient is asymptomatic.[13] The dental follicles present often with slight semicircular radiolucencies around unerupted teeth the width of which is of utmost importance in identifying dental follicular pathology. Studies have suggested that pericoronal radiolucency 2.5 mm or larger on standard panoramic radiograph may indicate an underlying abnormality. Indications for removal of third molars have generated much discussion in minor oral surgery. Some clinicians advocate removal before pathologic changes develop and periodic monitoring.[12] When the width of the radiolucent line exceeds, 3mm, it is believed to be pathological in nature while, if below 2.5mm, it is considered as normal.[2] However, scientific evidence supporting this assumption is limited and there is no internationally accepted consensus on the clinical criteria to differentiate between normal and pathological conditions based on radiographic features alone.[2] Recent studies have reported pathological changes in DF of upto 2.5mm with frequency varying from 23% to 58.5% in DF specimen associated with the asymptomatic third lower molar. Some clinicians found that in 53% of the all DF studied, DF which had thickness of ≤ 2.9 mm radiologically had developed pathological changes. The study had inherent limitation as the follicular tissue is

frequently discarded following extraction rather than being submitted for histopathological evaluation.

The type of pathology found in association with DF in different studies also varies. There had been reports of DCs as the only detected pathologic entity, while other authors have reported odontogenic keratocysts, calcifying odontogenic cysts, ameloblastomas, myxomas and odontogenic fibromas. Moreover a single case of low-grade fibrosarcoma in one such DF of an impacted third molar without any other clinical evidence was also reported.[2] A radiographic study by **Daley and Wysocki**[22] (1995) indicated an association between cystic changes and an impacted third molar in 77% of all studied cases.[4]

A study was conducted in 1999 to evaluate the incidence of histological abnormalities in soft tissues surrounding impacted third molar teeth when there were no pathological conditions apparent on the corresponding radiographs. In that study they collected 96 DFs from third molar teeth of 63 patients. None of the teeth had a follicular space greater than 2.4 mm as measured on the panoramic radiograph. A radiographic diagnosis of DC was made in 31 cases; 22 of 60 mandibular specimens (37%) and 9 of 36 maxillary specimens (25%) were affected. No lesion other than DC was diagnosed. They concluded that the incidence of DC associated with impacted third molarteeth is higher than reported in radiographic studies alone.[12]

Taiseer Hussain AK and **Batainneb AB** evaluated the incidence of radiographically detectable pathologic conditions around impacted mandibular third molars in Jordanians. Impacted third molars from 1398 patients were examined. Of this 46.4% of all third molars study showed associated radiographically detectable

lesions. All pericoronal radiolucent areas were histologically found to be either cysts or tumors. The most common cyst was DC while the most common tumor was ameloblastoma. Finally they concluded that high ratio of impacted tooth in the Jordanians groups had an associated pathologic lesions.[14]

Enlargement of perifollicular space in radiology or asymmetrical appearance can occur, which may be misinterpreted in imaging techniques. Pericoronal radiolucency is one of the most important factors to be borne in mind in the decision on whether to retain or remove an impacted tooth and the presence of radiolucency may be the only indication for this procedure. Few authors suggest that odontological lesions/conditions may be found even in minute follicular tissues and even in enlarged radiolucent areas there may be histologically normal tissues, so a biopsy is always imperative. Radiograph in most cases is the only instrument available to the clinician for deciding between removing or following up an asymptomatic unerupted tooth.[8] Evidence from a number of studies reveals the possibility of cystic degeneration and/or neoplastic transformation of the dental follicles in higher percentage indicates the prophylactical removal in case of strong suspicion.[15]

HISTOLOGICAL FEATURES:

Since 1948, there are studies reporting the pathological changes in an asymptomatic DF. **Khorasani & Samiezadeh[16]** in 2007 did a study to histopathologically evaluate follicular tissues of third molars with pericoronal radiolucencies of less than 2 millimeters. They analyzed 100 impacted third molars with normal follicular spaces which were removed and their pericoronal tissues submitted for histopathologic examination. Different characteristics of the epithelium

and connective tissue were evaluated in all cases. At the end of their study they did not detect any type of odontogenic or non-odontogenic lesion in their study sample. The only pathological change observed in their present investigation was non-specific chronic inflammation that showed a significant relationship with both squamous metaplasia and age. Few studies suggest that squamous metaplasia in pericoronal tissues of impacted teeth is related to normal changes that occur during aging while others believe this metaplasia to be an early pathologic event in DFs, possibly leading to cyst formation.[16]

Laura Villalba et al; [2] in 2011 performed a histomorphologic evaluation of the 140 DF. Of 140 tissues 123 of them (87.8%) exhibited epithelium on the surface. Reduced enamel epithelium was identified in 84 cases [78 cases of normal DF (NDF) (61.4%); 6 hyperplastic DF (HPF) (46.3%)], squamous metaplasia in 21 cases [17 NPF (13.4%); 4 HPF (30.8%)] and cystic epithelium in the remaining 18 cases [15 NPF (11.8%); 3 HPF (23%)]. The connective component was dense in 112 of cases [101 NPF (79.5%); 11 HPF (84.6)], 68 cases of presenting inflammation [58 NPF (45.7); 10 HPF (76.9%)],131cases of epithelial islands [118 NPF (92.9); 13 HPF (100%)] and 68 cases of presenting inflammation [58 NPF (45.7%); 10 HPF (76.9)].[2] The mesenchymatic component was analyzed to determine the type of connective tissue, that is, loose or dense, the presence or absence of inflammation and the presence of intramural epithelial cords-islands, and dystrophic calcifications. After performing histomorphologic analysis of the total cases, NPFs were selected to evaluate epithelial cell proliferation and apoptosis. Only NPF exhibiting no aggregate inflammation and sufficient epithelial length to enable assessment of at least 3 randomly selected microscopic fields were studied. Fourteen NPFs were obtained, 9 of which exhibited

reduced enamel epithelium and 5 showed squamous metaplasia and compared with 10 DC.[2]

Kotrashetti VS et al[13] in 2012 did a study on histopathologic changes in soft tissue associated with radiographically normal impacted third molars. This study was performed to histologically evaluate soft tissue pathosis in the pericoronal tissues of impacted third molars with pericoronal radiolucency measuring upto 2.5 mm on orthopantomographs. They analyzed 41 asymptomatic impacted third molars with follicular space of upto 2.5 mm of radiographs. The disimpacted teeth and the follicular tissues were obtained for histological examination. Of the 41 tissues evaluated, histopathological reports of 18 dental follicles were suggestive of DC, 2 dental follicles showed odontogenic keratocyst, 1 dental follicle each of calcifying epithelial odontogenic cyst, ameloblastoma- like proliferation, odontogenic myxoma and odontogenic fibroma. Twenty dental follicles showed presence of inflammatory component and dystrophic calcification within the stroma. 51.2% (21 dental follicles out of 41) of the dental follicles showed a cyst like change lined by stratified squamous epithelium. They advocated a thorough clinical and radiographic examination for all impacted third molars and stressed that the dental follicular tissue should always be submitted for histopathological evalution.[13]

In a large study involving 2646 PF lesions over a period of 6 years, 67% reported with no pathology while 28.42% reported with DC and a tenth of them exhibited metaplasia. The other pathologies included 71 odontogenic keratocysts, 19 odontomas, 13 ameloblastomas,6 carcinomas, 6 calcifying odontogenic cysts, 4 calcifying epithelial odontogenic tumours and 1 odontogenic myxoma.[17]

Damante JH and **Fleury RN**[1] verified the relationship between the radiographically measured width of the pericoronal space and the microscopic features of the small DCs and paradental cysts. One hundred and thirty unerupted and thirty partially erupted teeth were radiographed and extracted. The radiographic width of the pericoronal space of the specimens ranged from 0.1mm to 5.6mm. 68.4% of the unerupted teeth showed reduced enamel epithelium in the dental follicles. Inflammation was present in 36.1% of the cases, 20% showed stratified squamous epithelium and 13% showed no epithelium. In case of partially erupted teeth most frequent epithelium was the hyperplastic stratified squamous epithelium in found in 68.4% of the cases and reduced enamel epithelium in 68.5% of the cases. Inflammation was present in 82.8% of the cases. No epithelium was seen in 11.4% of the cases.The authors concluded that the final differential diagnosis between small DCs or paradental cysts and pericoronal dental follicle depends on clinical and or surgical findings, such as the presence of bone cavitation and cystic content.[1] **Listgarten MA**[18] using phase contrast and electron microscopy identified that the cells overlying the ameloblasts have two to six cells layers thick cells that were flattened and monomorphic. These cells were suggested to include cells of the stratum intermedium and outer enamel epithelium. It was observed that the REE near cemento-enamel junction was mostly continuous with the connective tissue of the dental sac. In certain areas, author identified split between the epithelial cells and the connective tissue. Citing the relatively constant finding, **Listgarten MA**[18] suggested that a weak region may exist in the connective tissue adjacent to REE. Using electron microscopy, attachment of the ameloblasts to adjacent enamel was identified as normally sized and structured hemidesmosomes on the enamel side of the cell membrane. A basal lamina

of 400° A wide structure was seen along the cell surface facing the enamel. This was continuous with organic structures along the enamel surface. The REE cell surface facing the basal lamina often had features suggestive of secretory or absorptive activity. There were vacuoles with cytoplasm of REE cell in close proximity to the cell surface.[18] **Nanci A et al**[19] used immunohisochemistry and identified numerous vesicles in such cells.[19]

DENTIGEROUS CYST

Dentigerous Cyst (DC) is the second most common type of odontogenic cysts. It is the most common developmental cyst of the jaws and is always associated with an impacted tooth. The cyst is more common in the first three decades of life with its peak in the third decade. The mandibular third molars followed by maxillary third molars and canine were the frequently involved teeth. There is a male predilection associated with DC in several studies. On the contrary large scale studies failed to identify such gender predilection for DC.[20]

Several authors attempted to induce odontogenic keratinizing cysts by transplanting maxillary first molar tooth germs from 2-day-old mice under the kidney capsules of isologous adult male mice. Nephrectomies were performed 1-180 days later after sacrificing the animal. They had observed that the tooth germs continued to lay down all tooth structures including induction of the formation of trabecular bone with heamopoietic marrow in the surrounding renal connective tissue. They also noted that clefts arose in the intermediate area of the reduced enamel epithelium, which was usually after cessation of enamel formation.They further observed that such clefts continued to enlarge until they enclosed the crown to produce the appearance of

typical DCs after about 50 days. In these cases, epithelial buds and multiple cysts were not uncommonly seen in some specimens and all cysts arose in reduced enamel epithelium. They reported that these cysts had epithelium that was flat, keratinized or parakeratinized with no rete pegs or inflammatory cells, the walls were not always as thin and the basal cells did not show the same columnar appearance of odontogenic keratocyst.[21] Comparing this finding with earlier reports of normal eruption pattern, the outer layers of the reduced enamel epithelium proliferate and unite with a downward growing knot of mucosal basal cells. This fused mass of cells encloses the erupting crown to form the so called "gingival cuff" and a split occurs in the reduced enamel epithelial component of the cuff, just above the degenerating ameloblast layer. It is this split which becomes the gingival crevice, normally, when the crown completely erupts into the mouth. By comparison, the experimental follicular cyst appears to arise in the same way as this programmed crevicular separation as reported. The enlargement beingsuggested due to the proliferation of the unbreached outer reduced enamel epithelium away from the immobile or retarded erupting tooth, so explaining the recognized relationship between follicular cyst formation and delayed eruption.[21] Some authors suggests that the likely origin of DCs is a breakdown of proliferating dental follicle cells owing to impeded eruption.[5] The pressure exerted by a potentially erupting tooth on an impacted dental follicle obstructs the venous outflow and thereby induces transudation of serum across capillary walls. Increase in hydrostatic pressure of these pooling fluid results in separation of dental follicle from the crown with or without decreased enamel epithelium. But all impacted teeth do not develop DCs.[5] The frequency of DC has been estimated to be 1.44 cysts for every 100 unerupted

teeth. Therefore, it was concluded that there must exist a decisive factor other than pressure that causes the cyst transformation.[5]

Daley TD and **Wysocki GP** [22] made an attempt to identify the epidemiological features that would assist in the differential diagnosis of DC and DFs. The study group comprised of 1662 DC and 824 DFs which showed considerable overlap in age distribution and site predilection and therefore were of minimal use in reaching a final diagnosis. Hence the authors concluded that identifying a cystic cavity at the time of surgery may be the only reliable way to arrive at a definitive diagnosis.[22] DC are currently diagnosed with all following features: i) Pericoronal radiolucency larger than 4 mm in greatest width as assessed on a panoramic radiograph ii) Histologically, fibrous tissue lined by non-keratinized stratified squamous epithelium and iii) A surgically demonstrable cystic space between enamel and overlying tissue, filled with fluid.[22]

According to the **WHO classification**, the DC is defined as a cyst, which encloses the crown and is attached to the neck of an unerupted tooth and develops by accumulation of fluid between the reduced enamel epithelium and the crown or between the layers of the reduced enamel epithelium. [23] The main location of the DCs is closely associated with the teeth in the dental arch that are most frequently unerupted. These are the areas surrounding the third molars, canines and premolars.[24] Mourshed reported a higher predilection for the mandible (74%). Histopathologically, odontogenic keratocyst and Adenomatoid odontogenic tumour were observed in DCs. As there is a danger of misdiagnosing keratocysts and Adenomatoid odontogenic

tumour in a DC location, it is important that the final diagnosis should be done using histopathological methods in addition to radiographic and clinical examination.[24]

Poomsawat S et al[25] compared the immunohistochemical expression of basement membrane components (BMCs), including laminins 1 and 5, collagen type IV and fibronectin in odontogenic keratocysts (OKCs), DCs and radicular cysts (RCs). They concluded that DCs demonstrated only one staining pattern of the investigated basement membrane proteins, whereas OKCs and RCs exhibited variable expressions. Staining pattern of DCs indicate an origin from one cell source and supports the fluid accumulation in REE pathogenesis. Unlike DCs, OKCs showed two distinct pattern of expression of basement membrane proteins, suggesting two different processes for their epithelial origin, different cell sources or the same cell type but with different stages of tooth development. This study concludes that OKCs, DCs and RCs did not demonstrate significant differences in the expression of laminins 1 and 5, collagen type IV and fibronectin.[25] In a study conducted in 2005 using immunohistochemical markers for apoptosis related factors such as Fas, bcl-2, single stranded DNA (ssDNA) to differentiate between DF and DC associated with impacted 3rd molars. This study was done to identify the role of pathogenesis of apoptotic related factors that transforms DFs to DCs. There was a statistically significant difference between expression of apoptosis related factors between DCs and DF. However, that did not correlate with clinical or radiological feature. This lack of sufficient correlation suggests that these features do not reflect the pathophysiologic nature of DF. Furthermore, they concluded that inflammatory changes play a vital role in upregulation of the turnover rate of DF.[25]

The difference of immunohistocehmical expression of various integrins in epithelial cell layers of DF and DC was studied by **Godoy GP et al**,[26] Significant difference existed in the staining pattern of DCs and DF with staining being more discrete in DF. Intense staining was more commonly seen in the DCs studied, especially in the basal cell layer of cyst. The difference was statistically significant. It has been earlier concluded that integrin α2 subunit plays an effective role in stratification of squamous epithelia, including the epithelium of cystic lesions. It is also shown that α2 integrin is expressed more in the basal cells of the epithelial wall of the DCs and odontogenic keratocysts. It is also shown that reduction of expression decrease in cell-cell adhesion and favors the desquamation of more superficial cells. Intense expression of α2β1 integrin in basal cells has been reported as compared with supra basal cells in the epithelium of DCs.[26]

Cases of oral squamous cell carcinoma arising from lining of DC have been reported in literature. It is reported that 25% of odontogenic origin of squamous cell carcinoma arise from DC.[27] However the exact nature, prevalence, molecular pathogenesis and prognosis of these lesions are undeciphered.[27]

ORAL MANIFESTATION OF RENAL PATIENTS:

Oral mucosa can be affected in many systemic diseases such as Chronic Renal Failure (CRF). Many metabolic and hematologic disorders develop in patients with CRF which also affect oral cavity. Oral mucosa, teeth, salivary glands and jaw bones are affected. Although hemodialysis can improve many of systemic features but the best treatment is renal transplantation.[6] Patients with CRF may present as unique signs of multi-system disease affecting the kidneys or as common oral pathologies found at

an increased prevalence in patients with CRF. The prevalence of oral lesions is affected by the accompanying systemic disease.[6] Chronic renal disease (CRD) is defined as a progressive and irreversible decline in renal function associated with a reduced glomerular filtration rate (GFR). The importance of CRD for the dental practitioner lies in the fact that an increasing number of patients with this disease will probably demand dental treatment and that up to 90% of them will show oral signs and symptoms related to this systemic disease.[7]

In these systemically compromised patients, total amount of saliva secretion is decreased, urea content is elevated which can decrease metabolic end products of bacterial plaque. This reduction results in increasing the buffering capacity of saliva and decreasing dental caries particularly in children, in spite of poor oral hygiene, high sugar content of diet and low-protein in this group of patients. Long-term corticosteroid therapy can implicate the situation. Due to frequent regurgitation and vomiting induced by uremia, medications and nausea related to dialysis severe erosions in lingual surfaces of teeth may occur. As buffering capacity and flow rate of saliva decreases, erosion develops.[6] Uremic stomatitis is a rarely reported oral mucosal disorder possibly associated with longstanding uremia in chronic renal failure patients. The clinical features of uremic stomatitis are poorly defined.[28]

ORAL MANIFESTATIONS OF CHRONIC RENAL FAILURE

(a) Gingival Enlargement

Gingival enlargement secondary to drug therapy is the most-reported oral manifestation of renal disease. It can be induced by cyclosporin and/or calcium

channel blockers. It principally affects the labial interdental papillae, although it can become extensive, involving the gingival margins and lingual and palatal surfaces.[29] The prevalence of gingival enlargement in individuals taking cyclosporine is unclear, and reportedly has a wide range-from 6 to 85%. [26] DCs have also been reported in patients taking cyclosporine and calcium channel blockers.[5]

(b) Oral Hygiene and Periodontal Disease

The oral hygiene of individuals receiving hemodialysis can be poor. For example, only 15% of 45 individuals receiving hemodialysis from 4 centers in Virginia, USA, had a good standard of oral hygiene. Deposits of calculus may be increased. There is lack of sufficient evidence of an increased risk of periodontitis although premature tooth loss has been reported.[29]

(c) Xerostomia

Symptoms of xerostomia can arise in many individuals receiving hemodialysis. Possible causes include restricted fluid intake, side effects of drug therapy and/or mouth-breathing. Long-term xerostomia may predispose to caries and gingival inflammation and can give rise to difficulties with speech, denture retention, mastication, dysphagia, sore mouth, and loss of taste (**Porter et al**[29]). It also predisposes to caries and infections such as candidiasis and acute suppurative sialadenitis.[29]

(d) Oral Malodor/Bad Taste

Uremic patients may have an ammonia-like oral odor which also occurs in about one third of individuals receiving hemodialysis. Chronic renal failure can give

rise to altered taste sensation, and some patients complain of an unpleasant and/or metallic taste or a sensation of an enlarged tongue.[29]

(e) Mucosal Lesions

A wide range of oral mucosal lesions, particularly white patches and/or ulceration, has been described in individuals receiving dialysis and allografts. In particular, lichenplanus like disease (sometimes termed lichenoid disease) can arise, sometimes, but not always, as a consequence of the associated drug therapy (e.g., diuretics, beta-blockers). Similarly, oral hairy leukoplakia can occur secondary to drug-related immunosuppression, although clinically and histopathologically similar lesions lacking Epstein-Barr virus (EBV) have been observed with uremia.[29] Uremic stomatitis may manifest as white, red, or grey areas of the oral mucosa. There are no good histological descriptions of uremic stomatitis; thus, it is difficult to define the cause of this unusual oral mucosal change. It has been suggested that uremic stomatitis may be due to chemically based trauma from elevated levels of nitrogenous compounds. In some instances, the mucosal surface may become erythematous or ulcerated. Oral mucosal macules and nodules have also been described in 14% of individuals receiving hemodialysis.[29]

(f) Oral Malignancy

The risk of oral squamous cell carcinoma in patients receiving hemodialysis is similar to that of otherwise healthy individuals. Although there have been reports suggesting that therapy following renal transplantation predisposes to epithelial

dysplasia, carcinoma of the lip, Kaposi's sarcoma and Squamous cell carcinoma have been reported.[29]

(g) Dental Anomalies

Delayed eruption of permanent teeth has been reported in children with CRF. Enamel hypoplasia of the primary and permanent teeth with or without brown discoloration, can also occur. Narrowing or calcification of the pulp chamber of teeth of adults with chronic renal disease can occur. The exact cause of this dental change is not known. Renal allograft recipients have significantly more narrowing of the pulp chamber than those receiving hemodialysis. There is no consistent association between corticosteroid therapy and narrowing of the pulp chamber.[29] Amelogenesis Imperfecta (AI) in renal disease patients was reported by **Elizabeth J et al** [30] who stated that AI may highlight the importance of renal functions in patients with AI. They concluded that a thorough medical history and systemic examination, including renal ultrasound and renal function tests is necessary in all patients with AI.[30]

Autosomal Dominant Polycystic Kidney disease:

The progressive formation and growth of renal cysts leading to end-stage renal disease (ESRD) characterizes a large spectrum of renal disorders known as Polycystic kidney diseases (PKDs). The most commonly inherited PKDs are passed on through one's genes as either autosomal dominant or autosomal recessive traits. Among the western population, autosomal dominant polycystic kidney disease (ADPKD) was reported by Wilson to occur with an incidence of about 1:1000 and was characterized by the development of fluid-filled cysts in the kidneys and other organs, accounting for up to 10% of ESRD cases[9]. Based on conservative estimates, the annual incidence of

ESRD in the Indian subcontinent is about 100 per million of population, which would translate to 100,000 new cases per year. **Sakhuja V**[8] have documented that 5% of all Indian ESRDs are caused by ADPKD[8].

The ADPKD is linked to two genes – i) PKD1 encoding for protein, PC1 mapping to chromosome 16p13.3 which has been mutated in 85% of all APKD instances while, ii) protein PC-2 gene, PKD2, mapped to 4q21 and is involved in 15% of the remaining cases.[8]

Structure of Polycystin-1

The amino acid sequence that was deduced from the cloned full-length cDNA of human PC-1 along with the hydropathy profiles was analysed, **Patrica D Wilson** [9] in her report predicted that PC-1 would be a large-membrane protein of about 4300 amino acids. The presence of a signal sequence led to the suggestion that the protein might be synthesized and processed through the secretory pathway of the cell. Hydropathy plot analysis suggests 9 to 11 transmembrane spanning regions. Using a variety of anti–PC-1 antibodies or epitope-tagged transfected cDNA or labeled proteins, many investigators have confirmed the prediction of membrane insertion of PC-1.[9] When PC-1 activation occurs at the cell membrane, it leads to intracellular signaling via the c-Jun terminal kinase and wnt pathways. This in turn leads to the activation of Activated Protein-1 (AP-1) and TCF/LEF-dependent genes, respectively. Both, the protein kinase A at S4252, and the focal adhesion kinase have led to the phosphorylysis of the C-terminal of polcystin-1 by c-src at Y4237. PC-1 focal adhesion complexes *versus* PC-1 adherence junction complexes is increased by increased cellular calcium, whereas disruption of the actin cytoskeleton dissociates all

PC-1 complexes. Genetic evidence suggests that *PKD1*, *PKD2*, and tensin are in the same pathway. [9]

PC-1, which is a large transmembrane protein with an estimated molecular weight of ~500 kD (Fig. 1)[11] (Annexure-VI), is the product of the PKD1 gene mapped to the human chromosome 16p13.3. It has two terminals – the N terminal region and the C terminal region. The large extracellular N-terminal region contains several specific motifs including leucine-rich repeats (LRRs), C-type lectin domain, LDL-A region, multiple Ig-like domains (or PKD domains), the REJ domain and the GPS domain. It has 11 transmembrane (situated along the membrane) domains, with a PLAT domain located in the first cytoplasmic loop and a small cytoplasmic tail with a protein- binding motif and coiled-coil region. The 16 Ig-like domains are segmented such that the first Ig-like domain is localized between the LRRs and the C-type lectin domain, while the remaining 15 Iglike domains are clustered together between the LDL-A and the REJ domains. This Ig-like domain plays a vital role in cell-cell adhesion. PC is a multifunctional protein with vital roles in cell-cell/ matrix adhesion and ciliary functions.[11] The protein PC-1 is a large 4302 amino acid protein described to have an extracellular region of approximately 2579 residues, multiple transmembrane domains, and a highly charged 225 residue cytoplasmic carboxy-terminus. The full-length recombinant identification has confirmed that the PC-1 possesses a mass of approximately 460 kDa, and a glycosylated mass of 520 kDa.[11] PC-1 undergoes partial cleavage at the GPS domain such that the N-terminal and the C-terminal polypeptides remain non-covalently linked for effective functioning. It is subsequently cleaved at the second site, which releases its C-terminal tail in to cytoplasm. On entering the nucleus, the cytoplasmic tail of PC-1 regulates cell

signaling events. There is a possibility that mechanical stimulus, such as ciliary beating, may initiate this signaling function of PC-1 that is regulated by PC-2. In primary cilia, PC-1 plays a role in calcium signaling, mechano and chemo-sensation, modification of signaling cascades (JAK-STAT pathway, Wnt pathway).[11] **Chapin HC**[31] proved that polycystins through Tuberous Sclerosis TSC1/TSC2 and mTOR (mammalian Target of Rapamycin) signaling cause decreased cell size, ribosome biogenesis and decreased protein synthesis. Through the STAT 1, 3 and Id2 pathway they interact with p21 to cause decreased cell growth and division. Through the G proteins, calcineurin, NFAT and AP-1, they cause modifications of differentiation, apoptosis and proliferation. Through the Wnt pathway they exert influence on gene expression and differentiation. The complete role of Polycystin has not been elucidated.[31]

PC-1 is proposed that to mediate cell-cell adhesion through PC-1 homophilic interactions of its Ig-like domains. Immunofluorescence studies have localized PC-1 with desmosomal junctions and adherence junctions besides PC-1. The components of desmosomal junction include: desmoplakin, desmogleins, desmocollins and plakoglobin. The adherence junction usually consists of E-cadherin as well as other types - a, b and g-catenins. The adherence junction complex provides PC-1 a linkage to the actin cytoskeleton, and the desmosomal junction interacts together with the intermediate filaments of the epithelial cells. Quite often, in ADPKD epithelial cells, dysfunctional PC-1 and hence desmosomal proteins are lost from cell-cell contacts and remain in the intracellular vesicles. In addition, there is a reduction of E-cadherin expression, which results in the compensatory expression of N-cadherin (N-cad). Thus, quite often, there is disruption of the adherence and desmosomal junctions in ADPKD

cells, while the tight junctions only remain intact. In normal epithelial cells, PC-1 is found in a complex aggregation with talin, paxillin, vinculin, focal adhesion kinase, c-src, p130-cas, nephrocystin and tensin. In ADPKD cells, expression of FAK is lost from the focal adhesion complex, that leads to incomplete adherence. According to **O. Ibraghimov-Beskrovnaya,**[11] it is with the talin moiety that integrins (both alpha and beta) interact.[11] **Xu GM**[32] also stated that through the intracellular and extracellular domains, PC-1 interacts with the epithelial intermediate filaments.[32]

According to Hogan MC, exosomes are smaller, intracellular, cytoplasmic vesicles that are 50 to 100 nm in diameter and are considered a source of biomarkers for various renal diseases. The exosomes that exist in kidney cells, namely, the urinary exosomes are thought to be end products of the multivesicular body (MVB) sorting pathway in which these membrane proteins are uniquely packaged into intraluminal vesicles (ILVs) within the MVB, some of which are secreted as exosomes when MVBs fuse with the apical plasma membrane. Using extensive proteome analysis, **Hogan MC et al**.,[33] in 2009 has characterized the APKD urinary exosomes and identified 552 proteins of which 232 have not been characterized in urinary proteomic databases. Of these proteins, many have been implicated in signaling. PC-1 has been also expressed in these exosomes.[33]

Tissue Localization of Polycystin

PC-1 is expressed in almost all of the epithelial cells, and in a variety of somatic tissues including the heart, the liver, bones, and the endocrine glands. Expression of PC-1 is temporally regulated, with the highest levels found in the fetal tissue and low, but detectable levels present in the adult tissue. The subcellular localization of PC-1 has been most extensively studied in renal epithelial cells and

multiple different locations of the proteins have been identified. PC-1 expression is observed at several areas, including lateral membrane junctions (tight junctions, adherens junctions, desmosomes), focal adhesions, apical vesicles, and primary cilia in varying quantities. This distribution indicates a complex picture that functionally, PC-1 is developmentally regulated and suggests that the proteins may rarely act independently. **Zatti et al**, [35] reported that PC-1 is expressed in the plasma membrane, and its related protein, PC-2 in the endoplasmic reticulum does not preclude interaction, and has described the coupling that exists between TRP channels in the plasma membrane and inositol-1,4,5 triphosphate (IP3) receptors in the adjacent endoplasmic reticulum.[35] PC-1 is found in the cilium, but, in polarized epithelial cells, it is also found to localize to the lateral domain of the plasma membrane and adhesion complexes.[35] The presence of polycystin in epithelial plasma membranes indicates that the extracellular matrix abnormalities noted in ADPKD are not likely to be primary defects. It has also been suggested that the PC-1 is directly involved in the sorting of membrane proteins. In patients with ADPKD, a variety of membrane proteins, including Na1, K1- ATPase, fodrin, and the epithelial growth factor receptor are abnormally localized within cyst-lining cells. As PC is primarily present in cell surface plasma membranes, it is highly unlikely that PC is a sorting protein, but rather has a primarily developmental function.[36]

Chae SW et al [37] have pointed out that certain controversies have risen regarding the PC-1 expression when using different antibodies to detect it. These difficulties with respect to studying PC-1 are mainly due to the antibody specificity and the low levels of PC-1 expression in cells and tissues. [37]

According to reports by **O. Ibraghimov-Beskrovnaya** [11], polyclonal bodies against the LRR region near the N terminal of PC- 1exhibited the following: PC-1 expression was not observed in sections from the esophagus, the small intestine, the colon, or the rectum. Staining of the cervix and the skin demonstrated PC-1 expression in both the squamous epithelia and the endothelial cells. In normal brain tissue sections taken from juxta-tumor region, PC expression was identified in astrocytes of the temporal neocortex while the same cells were negative in the frontal cortex and the cerebellum. Weak staining was also observed in the vascular endothelium of surface vessels. In all the tissue sections that were studied, excluding the brain, cytoplasmic staining was also accompanied by a clear and consistent membrane accentuation. In ADPKD Kidney, PC-1 expression was seen in the epithelial lining of the cyst. Although staining appeared more intense than in normal tissues, it was variable even within sections and within cysts. Some of the cysts were also negative. In ADPKD, cyst staining appeared to be predominantly cytoplasmic, but areas of membrane staining were also observed.[11]

Peters DJM et al [38] used several polyclonal antibodies for specific regions of PC-1, in several human tissues and Madin Darby canine kidney (MDCK) cell lines. They reported that the salivary gland epithelial lining of the intercalated, striated, and large excretory ducts were positive. Staining was weaker in the serous secretory acini of the combined mucous and serous secretory gland. Mucous acini and adipocytes were found to be negative.

In the epidermis, stratum basale, stratum spinosum, and stratum granulosum were positive with variable intensities in different tissue specimens. In the dermis, there was staining in the epithelium of the hair follicles (root sheath) and the sebaceous

gland, and in the sweat glands.[38] The reactivity for other tissues is given in Annexure-VI.

Palsson et al [36] reported that PC-1 is expressed in many types of cells involved in the secretion or transport of molecules and highly organized cells with selected membrane domains involved in specific functions. It was often observed to express itself in tissues that were subjected to great mechanical pressure and often expressed a high number of adhesion molecules including the skeletal muscle, the heart muscle, cardiac valves, blood vessels, and the skin. They speculated that the expression pattern suggests a role for PC-1 in transporting (in epithelial and endocrine) cells and tissues which are subjected to increased mechanical pressure.[36] As PC appears to be a developmentally regulated and membrane-associated glycoprotein, its intracellular localization in the cyst-lining epithelium of ADPKD kidneys, suggests an abnormality in protein sorting in this disease. PC-1 is increased in ADPKD cyst-lining kidney epithelium. Its subcellular localization is abnormal, being largely restricted to the cell cytoplasm. [36]

It also has been demonstrated by **Bukanov et al** [39] that the status of PC-1 activity is different in normal tubules (strong basolateral) versus cysts (diffuse cytoplasmic). This change or difference in PC-1 expression in tubules versus cysts could only be explained as its redistribution from the basolateral location in the normal state to cytoplasmic in the cystic state. Alternatively, the lack of PC-1 at the basolateral site of cystic cells can be due to the general downregulation of its expression.[39] Subcellular localization of PC by **Xu-GM et al** [32] found PC-1 to be a component of various cell junctional complexes, intermediate filaments to be

associated with the cytoskeleton, but the specificity and nature of such associations are not known. [32]

The role of PC-1 in molecular cystogenesis has not been defined. The analysis of temporal and spatial patterns of PC-1 expression in normal and cystic tissues suggest an important role for PC-1 in epithelial cell differentiation and maturation.[39] PC-1 expression has been identified in several epithelial cells, renal tissues and other tissues in the human body. **Qian Q et al**.,[40] have analyzed and characterized the pattern of expression, subcellular localization, and interaction of the PCs in vascular smooth muscle cells. They identified the location of some PC-1 products on the plasma membrane using Immunofluorescence and Immuno-gold electron microscopy.[40]

Battini et al [41] reported that suppression of PC-1 was being associated with the increased expression of integrin α2β1 and reduced apoptosis in cells grown on collagen type I. Integrin α2β1 is essential for the survival of PC-1, because PC-1 knockdown cells are significantly less susceptible to anoikis by a mechanism that was reversible by anti-integrin α2β1 blocking antibodies. The apoptotic pathway is triggered by the loss of cell anchorage, through a mechanism that involves integrin α2β1. An imbalance with its stoichometric interaction factors could be caused by the fluctuations of protein levels above or below the normal thresholds thus leading to a cystogenic signal. [41]

It has been proposed by **Weston BS** [42] that PC-1 exists in at least two distinct membrane locations. If the PC-1 C-type lectin and/or LRR domains associated with ubiquitous extracellular matrix molecules *in vivo*, and PC-1 co-localises with beta2 alpha1 integrin in focal clusters, then PC-1 must exist in the basal membrane under

ideal circumstances. On the other hand, PC-1 must also exist in the lateral membrane if it is to co-associate with E-cadherin, the catenins and desmosomes as described.[42]

The difficulties of studying PC-1 are due to the antibody specificity and the low levels of PC-1 expression in cells and tissues. It has been reported that in the early fetal kidney, PC-1 was localized to the plasma membranes of the ureteric buds and the S-shaped bodies. However, in the late fetal kidney and in the ADPKD kidney, the majority of PC-1 staining was intracellular.[37]

The subcellular localization of PC-1 is mainly on the cell surface membrane and it may change in some conditions. Studies have shown identical patterns of PC-1 expression for the fetal, adult and ADPKD kidneys with immunofluoroscent staining, but immunohistochemical studies showed a cytoplasmic staining pattern of PC-1. Hence, it has been concluded that these PC-1 antibodies are sensitive, but there does not exist any specific technique to detect them, the reason being, that the epitope recognized by these antibodies may be not the full length, but only a fraction of the protein. [37]

Antibodies directed against the amino (N) as well as the carboxy-terminus (C) of PC-1 demonstrate that the endogenous protein is expressed in the primary cilium and co-localizes with beta-tubulin IV, a known component of the ciliary axoneme. PC-1 has been detected in the cytoplasm as well as exhibits apparent increased signal intensity at the lateral membrane, just below the level of the tight junctions. The PC1-FP-LRR (including 7e12 region) antibody, generated against a fusion protein containing residues 27 to 360 of the human PC-1, detected the protein in the cytoplasm as well as in the lateral membrane of cultured human proximal tubular cells and co-localization with desmoplakin in MDCK cells.[43]

Scheffers MS[44] reported that those motifs that stained with antibodies generated to the LRR domain (7e12) or to multiple N-terminal domains (NT2B) had no effect on cell adhesion. This finding was explained by providing three major reasons, each having functional implications. Firstly, although there are likely to be multiple epitopes recognized by the used polyclonal antibody, these sequences may not be critical for either homophilic or heterophilic PC-1 interactions unlike those recognized by IgPKD. Secondly, PKD domains (II-V) have the lowest affinity homophilic interactions *in vitro* and so they may be less critical for mediating intercellular adhesion. Thirdly, it is possible that NT2B could preferentially recognize epitopes in its denatured rather than in its native state. [44]

Chapin HC et al [45] suggested that the localization of PC-1 in cytoplasm as well as membranes could be a reflection of the precursor pool of PC-1 that transported towards the cell membrane as observed during Ca^{2+} switch or, it could mean a different reserved function for PC-1 in different cellular compartments at different phases of cellular differentiation[45]. For an effective function, membrane proteins must be targeted to specific locations and be restricted to specific subcellular locations. To enable this, eukaryotic cells have developed intricate signaling pathways and cellular trafficking machinery to guarantee the establishment and maintenance of these cellular localizations. Mislocalization of essential proteins can cause unfavorable results at the cellular and tissue levels. There are very few genetic diseases such as ADPKD, that characteristically alter a particular protein's distribution.[31] PC-1 undergoes several cleavages, post formation, during processing and movement through the secretory pathway. One such cleavage occurs at a G protein-coupled receptor proteolytic site (GPS) [along the extracellular N-terminal domain] near the first transmembrane

domain. Cleavage at this particular site involves a cis-autoproteolytic event that occurs early in the secretory pathway. Missense mutations in polcystin-1 genes, which may be normally essential for autocatalytic GPS cleavage would impair the normal functions of the PC-1 protein, as reflected in the formation of ADPKD[31]. It has been identified that in the early secretory pathway, GPS cleavage occurs before PC-1 arrives at the plasma membrane. When such cleavages are prevented, PC-1 N - Terminal Deletion (NTD) constructs are retained inside the endoplasmic reticulum and do not reach the plasma membrane. The intracellular release of PC-1 from the endoplasmic reticulum is not due to the release of polcystin-1 from associations with chaperone proteins upon polcystin-2 binding to form a heterodimeric complex with PC-1, a mechanism that regulates the surface delivery of other similar multimeric membrane protein complexes. Rather, PC-2 acts on PC-1 early in the secretory pathway to promote the cleavage of polcystin-1 at the GPS site, leading to its subsequent delivery to the plasma membrane. Based on the cleavage at the GPS site, PC-1 in the intracellular pool is divided into two functionally distinct groups – one that reaches the cell membrane and the other that does not. PC-2's effects on the C- and N-terminals of PC-1 cleavages may lead to distinct pools of PC-1 protein that ultimately reside in different subcellular compartments and, as a result, serving functionally distinct roles. In order to study the co-expression of PC-2 that produced abundant PC-1 surface delivery, antibodies made against PC-1-NTD lacking a majority of the extracellular N-terminal domain were used. There was little or no PC-1 NTD detectable at the plasma membrane. It is possible that the NTD mutations cause misfolding of the PC-1 protein or otherwise impair its trafficking through mechanisms independent of any effect on GPS cleavage. It is safe to conclude that the essential role of cleavage in PC-1 surface

delivery is not attributable solely to the physical dissociation of some putative transport-inhibiting component residing in the PC-1 N-terminus from the C-terminal fragment. This cannot be attributed to PC1 NTD lacking most of the N-terminal fragments, but rather it failing to reach the plasma membrane when co-expressed with PC-2. [31]

FUNCTIONS OF POLYCYSTIN-1

Chapin HC et al [45] reported that in order to elucidate the function of PC-1, several approaches have been attempted. These include determining the expression levels of mRNA and protein, tissue, cellular and subcellular distribution patterns and biochemical and molecular analyses. [45]

PCs through Tuberous Sclerosis TSC1/TSC2 and mTOR (mammalian Target of Rapamycin) signaling leads to decreased cell size, ribosome biogenesis and protein synthesis. Through STAT 1, 3 and Id2 pathway, they interact with p21 to cause decreased cell growth and division. Through G proteins, calcineurin, NFAT and AP-1, they cause modifications of differentiation, apoptosis and proliferation. Through the Wnt pathway they exert influence on gene expression and differentiation. The complete role of PC has not been elucidated in all human tissues. [45]

The wild type of PCs and the mutated ones exhibit different characteristics. Phenotypically, the wild type of PCs cause renal epithelium cells to be well differentiated, polarized, with normal cell- cell/matrix interaction, with a low rate of division and apoptosis, has good reabsorptiveness and normal urine concentrating capacity. The mutated ones are de-differentiated, with polarized defects, increased cell-cell/matrix adhesions, high rate of division and apoptosis, with reduced concentrating capacity and have a secretory phenotype. [46]

Weston BS et al[42] stated that Leucine -Rich repeats (LRR) have been proposed to mediate between protein-protein interaction and cellular adhesion. The cell wall integrity and stress component protein (WSC) domain and C-type lectin domain are predicted for a calcium dependent carbohydrate binding. The Low Dentisty Lipoprotein (LDL-like domain) like domain has a part to play for the extracellular part of the protein to turn hydrophobic with the potential ligand binding site. The Polycystic Kidney Disease (PKD) repeats are Ig like folds that indicate that these domains may be associated with protein-protein and protein-carbohydrate interactions. The sea urchin Receptor for Egg Jelly (REJ) domain supports the calcium ion influx in association with the protein. G protein-coupled receptor proteolytic site (GPS) motiff is just at the area of cell membrane. A recent study has now shown that PC-1 is indeed cleaved most likely at the GPS domain, a process that requires the adjacent REJ module to be present. Cleavage occurs rapidly after synthesis, although most of the N-terminal cleaved fragment remains at the cell surface. Known ADPKD mutations in the REJ module prevent cleavage and signal transduction through PC-1, leading to the suggestion that cleavage is vital for PC-1 to exhibit full biological activity, perhaps through the creation of a novel ligand-binding pocket. [42] PKD repeats are involved in mediating both cell-cell contact. The PC-1 LRRs inhibit cell proliferation *in vitro* indicating that the extracellular portion of PC-1 is important for PC-1 function. Moreover, it has been recently demonstrated that mouse PC-1 and PC-2 located in the single non-motile primary cilium on kidney epithelial cells are important for calcium influx in response to physiological fluid flow. When a blocking antibody raised against the first PKD domain of PC-1, it abolished this activity, leading to the suggestion that the N-terminal portion of the PC-1 protein may act as a mechano-fluid

stress sensor, thus providing further evidence that this region is functionally important, at least under certain circumstances. [42]

Expression

The tissue, cellular and subcellular distribution of proteins provide a first line of interpretation of putative protein function. [9] Although the exact function of these two proteins has not been fully elucidated and enumerated, there appear to be at least four membrane effects:

1. Activation of PC-1 causes activation of PC-2 and release of Ca^{2+} from the endoplasmic reticulum into the cytoplasm.
2. PC-1 can also cause the entry of extracellular Ca^{2+} via the cell membrane PC-2.
3. PC-1 activates heterotrimeric G-proteins, thus affecting the activities of adenylyl cyclase, MAP kinases and other effectors that can effect fluid secretion, proliferation and differentiation.
4. PC-1 can induce cell cycle arrest via activation of the JAK-STAT signalling pathway.

Activation of PC-1 and PC-2 appear to cause an increase in intracellular Ca^{2+} levels, thus causing a reduction in cAMP via direct inhibition of cAMP and stimulation of phosphodiesterase activity, which metabolizes cAMP. They also have an anti-mitotic effect, that causes cell cycle arrest. [10]

MUTATIONS

ADPKD is caused by mutations in the PKD1 or PKD2 gene. PKD1 and PKD2 genes encode proteins PC-1 and PC-2, respectively. Both proteins have been recently detected on the primary cilia that are present on most of the epithelial cells in the

nephron. Mutations in the PKD1 gene on chromosome 16 is responsible for 80% of cases of ADPKD patients, whereas in 50% of the cases, mutations on the PKD2 gene on chromosome 4 are responsible for ADPKD patients. Germ line mutation usually occurs in only 1 allele of the PKD1 or PKD2 gene for ADPKD patients. The remaining normal allele is sufficient for all developmental functions, because patients exhibit no developmental anomalies. Renal cysts in ADPKD arise after spontaneous second mutations that affect the normal allele, which result in cells devoid of a functional gene. PKD1-mutated patients are clinically indistinguishable from PKD2-mutated patients, but PKD2 patients have a less severe course of the disease. [5] The kidneys are grossly enlarged with multiple cysts studding the surface of the kidney. The cysts contain straw-colored fluid that may become hemorrhagic. The presence of multiple epithelial-lined kidney cysts result in gradual kidney enlargement and subsequent kidney failure in the majority of individuals. The penetration of the disease is 100%. ADPKD patients also exhibit extrarenal manifestations that are characterized by cysts in different organs, such as the liver, the pancreas, and the ovary[5]. The PKD1 gene is complex with 46 exons that generating a large transcript containing a long open-reading frame and is predicted to encode a 4302aa protein. This renders the characterization of the PC-1 gene structure and identification of mutations complex. The genomic duplication of the 5' region of PKD1 (to exon 33) is such that approximately six copies of PKD1-like genes, with various rearrangements relative to PKD1, are located in 16p13.1. This makes the number of combinations of mutations large. Besides these, the sequence similarity of pseudogenes (many of which encode transcripts but probably not significant active PC-1protein products) to PKD1 mean that specific anchored, long and locus-specific amplification methods are required to

characterize and screen PKD1 for mutations. These factors together cause marked allelic heterogeneity, with approximately 200 different PKD1 mutations. The majority of these mutations are unique to a single family, passing a same type of mutation, thus illustrating that a significant level of new mutation is occurring. Most mutations are predicted to truncate the protein (due to frame-shifting deletion/insertion, nonsense changes, or splicing defects), but a significant level of in-frame and missense changes have also been described. Mutations of PKD1 are found in the 3' half of the gene. This pattern of mutation is consistent with the inactivation of one allele but recent genotype/phenotype correlations in PKD1 suggest that not all mutations may have the same phenotypic outcome. In PKD1, mutations of 5' to the median are associated with a more severe form of the disease. This association is not directly related to mutation type but may be due to the proposed cleavage of PC-1 into two different proteins, with mutations to each half having potentially different phenotypic consequences[34]. It has been reported that both, over expression and loss of PC-1 result in renal cyst formations in rats[48]. Over expression of PC-1 still results in renal cystogenesis but they have a very milder course. The increased immunoreactivity of PC-1 in ADPKD cyst epithelia is probably due to the loss of functional phenotype with increased PC-1 expression. In simpler terms, there is an overexpression of a mutant PC-1 that is unable to function sufficiently.[48]

Ong ACM [34] reported that a negative expression for antibody to PKD1 region in ADPKD cysts were also negative for the LRR antibody [7e12]. As the LRR antibody in theory, detects mutant PCs-1 proteins, the absence of staining in the study by **Ong ACM**[34] indicates that PC-1 may become functionally unstable when truncated.[34] The severity of the disease in ADPKD has been directly associated

with the gene that was mutated. The complete phenotypic spectrum, however, ranges from rare in utero–onset cases to patients with adequate renal function in old age to extreme heterogeneity that cannot be solely explained by genic effects. Some rare cases with early-onset ADPKD also develop tuberous sclerosis due to a contiguous deletion of PKD1 and TSC2 gene. There are reports that non-genetic factors, such as male hormones, caffeine, and smoking, have been suggested as risk factors for ADPKD.[48]

Malignant Potential of ADPKD

There are many reports in various literature of instances of renal cell carcinoma arising out of long term PKD and ADPKD cystic lining. Even though a coincidence of occurrence or casual occurrence is rare, it cannot merely be attributed to chronic inflammatory responses seen in these disease conditions. Such discussions have been reported by **Na KY et al**.,[49] from Korea. They concluded that large scale studies are essential to identify the exact, prevalence as well as significance of these neoplastic changes. [49]

Primary Cilium

The primary cilium acts as a sensory organelle to transfer information from the extracellular environment to the cell's interior. [5] It also acts as a mechanosensor through proteins PC-1 and 2. Receptors such as platelet-derived growth factor on the primary cilia bind to extracellular ligands and activate the Akt and Mek1/2-Erk1/2 pathways that control cell proliferation, migration, and apoptosis. It regulates crucial cellular processes such as cell cycle, cytoskeletal organization, and intraflagellar transport (IFT). Two important signaling pathways are also associated with primary cilia, namely, the Wnt pathway and the Shh pathway[5]. Primary cilia play a crucial role

in mediating hedgehog signaling in vertebrates. Shh is a member of the vertebrate hedgehog signaling proteins. Ptch is the receptor for the Shh ligand. In the absence of Shh, Ptch localizes to the cilium and prevents Smo (Smoothened), a transmembrane protein from moving into the cilium. When Shh binds to Ptch, a complex is formed that moves into the cytoplasm. Smo moves into the cilium and processes the transcription factor Gli (glioma-associated oncogene homolog). The transcription of *SHH* target genes is regulated by the nuclear ratio of Gli activators to Gli3 repressors. Gli activators translocate to the nucleus where they activate transcription of *SHH* target genes. In the absence of Shh, the cilium-mediated proteolytic processing of Gli3 generates an N-terminal fragment that functions in the nucleus as a transcriptional repressor. Recently, two cilium proteins, PC-1 and 2, also have been found to localize in the primary cilium. [5]

Primary cilia are cell organelles originating from the basal body and are related to the centriole. Their basic structure consists of a central axoneme composed of nine doublets of microtubules and a ciliary membrane continuous with the plasma membrane. One or two primary cilia are present on the surface of most vertebrate cells. With the exception of intercalated cells, every epithelial cell lining the nephron carries at least one primary cilia, which is usually long (2–30 mm) and thin (0.25 mm in diameter). Renal primary cilia protrude into the tubular lumens and are in contact with the urine. Although they may have some motility in lower vertebrates, renal primary cilia in mammals do not propel urine. The prevailing view is that the primary cilia is a vestigial structure from a motile ancestor and is hence totally useless for the cell. But, this hypothesis was recently challenged by the fact that several proteins

implicated in the biology of polycystic kidney diseases were located in the renal cilia.[50]

It has now been confirmed by several groups that PC-1 and PC-2, along with polaris, cystin-1 and inversin, are co-localized in the primary cilia of renal epithelial cells. PC-1 may localize at different cell membrane compartments, such as the cell–cell lateral contacts or at the basal pole of cells. What will be of particular interest will be the determination of the link between the cilia, the PCs and the main PC-1 dependent signalling pathways identified so far, that include the JAK2-STAT1-p21 and the Wnt-catenin pathways. [50]

CILIA AND CYSTOGENESIS

According to **Dominique Joly et al,**[50] according to the APKD 'double-hit theory', the initial and critical molecular event underlying cyst formation is the occurrence of a 'second hit' (a somatic PKD1 or PKD2 mutation), which super imposes on the initial germinal PKD1 or PKD2 mutation that has been inherited. This theory accounts for the focal nature of cysts, but does not explain why tubular cells dedifferentiate, proliferate and ultimately form continuously growing cysts. In mice, that had a homozygous invalidation of both polycystin-1 and 2, the first renal cysts were detectable after 15–16 days of gestation, i.e. after the onset of a glomerular filtration flow. This observation indicates that polycystins are not mandatory for the initial steps of tubulogenesis, but also suggest that mutated PCs may fail to transmit to the tubular cells a 'stop signal' normally generated by the urinary flow. Tubular cells unable to perceive the ciliary 'stop signal' may become hyper proliferative, dedifferentiated and initiate an aberrant tubular growth leading to the formation of a cyst. Thus, if fluid-flow perception controls the diameter and differentiation state of

renal tubules through cilia, any anatomic and/or functional anomaly of cilia (induced by mutations of polycystins, polaris, cystin or inversin) may induce renal cystogenesis. Altered perception of cilia bending may also account for other clinical features of PKD, such as liver and pancreatic cysts, colonic diverticuli or vascular aneurysms. The identification of cilia dysfunction as a key feature of polycystic kidney diseases will also encourage new therapeutic strategies. For instance, taxanes, which promotes microtubule assembly (one of the putative functions of cystin), were shown to ameliorate renal cystic disease in mice. Hence, to speculate that any other pharmacological agent might be able to improve tubular ciliary functions or to stimulate PC complex mechanoreceptors will be a strong candidate for PKD treatment.[50]

When PC-1 is suppressed, it is associated with an increased expression of integrin α2β1 and reduced apoptosis in cells grown, *in vitro* on collagen type I. Integrin α2β1 is essential for the survival of PC-1, because PC-1 knockdown cells were significantly less susceptible to anoikis by a mechanism that was reversible by anti-integrin α2β1 blocking antibodies. **Battini L et al**[41] reported that PC-1 knockdown cells seem more resistant to anoikis. Through a mechanism involving integrin α2β1, the apoptotic pathway is triggered by the loss of cell anchorage. In order to maintain normal renal tubule dimensions, it is believed that PC-1 needs to express within a specific range. If protein level fluctuations is above or below normal thresholds, its stoichiometric interaction factors will become unbalanced, thus leading to a cystogenic signal. [41]

PRIMARY CILIA AND ADPKD:

The primary cilium is considered to be an important signaling center within the cell. It is present in almost all the cells of the human body. As far as ADPKD is concerned, cliometrician and chemical defects in primary cilia have been observed. With regard to PKD, the mutation of PC-1 and PC-2 genes which also has been localized to ciliary structures has been identified. Studies have identified localization of the PC-1/2 complex to primary cilia and its role in mediating Ca^{2+} fluxes in response to mechanical stimulation. A primary cilium, with few exceptions, decorates the apical surface of the renal tubular epithelial cell. Primary cilia in cell cultures of MDCK cells function as mechanosensors and chemosensors that translate mechanical or chemical stimuli (fibronectin–β-integrin interaction)[41] into Ca^{2+} fluxes. Cells isolated from mice that lack functional PC-1 have their Ca^{2+} influx in response to physiological fluid flow perturbed. This same phenomenon is also observed in wild-type cells that have been treated with antibodies against the extracellular domains of PC-1 or PC-2. Cells with only one PKD germline mutation reportedly detect luminal fluid shear with sensitivity similar to that of wild-type cells. Antibodies directed against an extracellular epitope of fibrocystin also abolish the Ca^{2+} response to flow stimulation. The exact intracellular calcium homeostasis mechanism has not been described till date. Several theories and mechanisms have been postulated, but none has been proven till date. However, it has been clearly demonstrated that any such intracellular calcium modulation is mediated through the primary cilium from external sources. Hence, ADPKD has also been described as a ciliopathy.[46]

DENTIGEROUS CYST AS A CILIOPATHY

DCs are normally associated with an impacted tooth. In normal individuals, the pressure from the erupting tooth are sensed by the primary cilium in the reduced enamel epithelium (that covers the tooth), leading to an influx of calcium, which decreases cyclic adenosine monophosphate (cAMP) in the cells and maintains the cells in quiescence. This increase in cAMP leads to the proliferation of cells that are prone to cyst formation. Hence, an abnormal calcium response in REE prone cells will promote cystogenesis and which could play a role in the formation of DCs. This hypothesis is supported by the fact that the loss of function of Polaris has been found to affect Intra Flagellar Transport (IFT) in both renal and odontogenic cells[5]. IFT mutants have a stunted primary cilium and an abnormal calcium response in renal cells. Individuals who exhibit IFT mutations, see an abnormal calcium response due to a defective primary cilium when induced by drugs which may also lead to increased cAMP in the cells, resulting in cellular proliferation. Cyclosporine induced bilateral DCs in a patient stands as proof of this hypothesis. More studies are needed to investigate this phenomenon. The mutated REE cells may also secrete an abnormal extracellular matrix that may form a basement membrane like kidney cysts.[5] The basement membrane may attach to the cementoenamel junction (CEJ) as well as to the surface of enamel. So the cyst can enclose the tooth and also line the surface of the enamel. The recent hypothesis of DC as a ciliopathy has been put forward with the demonstration of the primary cilium in the lining cells of DC which further strengthens the concept.[5]

Thirty one Dental follicles that were clinically, radiologically and histopathologically asymptomatic were obtained from 30 cases. Of this 30 cases, 9 (29%) were males and 21(71%) were females (71%). Age of these patients ranged between 16 years to 48 years with the mean of 25.5 ± 8.09 years. DF tissues of 8 cases (26.6%) were associated with maxillary 3rd molars and 22 (73.3%) with mandibular 3rd molars. One case was collected from mandibular canine. Clinically 15 of all cases (48.4%) were associated with inflammation. Partial impaction of teeth was observed in 8 cases (25.8%). All partial impacted teeth were mesioangular type of impaction and fully impaction is seen in 23 cases (74.2%) (Table 1, Graph 1). Duration of the clinical inflammation ranged from 1 week (0.25 months) to 12 months the mean of 3.07 months. In DC (n =15) cases, there were 11 males (73.3%) and 4 females (26.7%). The mean age of patients ranged from 12 years to 57 years with a mean of 36.8± 15.02 years. The mean age of males was 39.27 ± 15.3 while for females it was 30 ±13.74 years. The difference was not significant. (P = 0.307)

Five micrometer thick, Hematoxylin and Eosin stained histological sections were prepared from such DF tissues after careful removal of tooth using techniques mentioned by Kotrashetti et al.[13] Sections were observed under light microscope using low and high power. Histopathological observation revealed, absence of inflammation in 13(41.9%) DF tissues, chronic inflammatory cells in 9 (29%) DF and mixed inflammatory cell infiltrate in 9 (29%) DF tissues. On studying the localization of inflammatory cells, diffuse presence of inflammatory cells were seen in 6 cases (19.4%) and localization of inflammatory cells were seen in 12 cases (38.7%). (Graph 2A,2B)

Parakeratinized stratified type of epithelium was observed in 7 cases (22.6%), ciliated epithelium was seen in 1 case (3.2%) and columnar epithelium was seen in 1 case (3.2%), Non-keratinized stratified epithelium was identified in 19 cases (61.3%) and REE type of epithelium seen in 3 cases (9.7%) (Graph 2C). Of all DC, 6 cases (40%) had stratified parakeratinized and non-keartinized type of epithelium while 9 cases (60%) had only REE type of cystic lining.

In the DF connective tissue, odontogenic rests were observed in 4 cases (12.9%), calcification was seen in 8 cases of which 6 cases (19.4%) were eosinophilic in nature and 2 cases (6.5%) were basophilic in nature. Association with vital bone was seen in 5 cases (16.1%). The connective tissue was described as fibrous in 28 tissues (90.3%) while it was normal in 3 tissues (9.7%). Giant cells were observed in 2 instances. (Table 2).

Clinically, radiographically and histopathologically confirmed, asymptomatic 31 DF and 15 clinically, radiologically and histopathologically confirmed cases of dentigerous cyst (DC) were stained with PC-1, immunohistochemically and studied. (Table 6, Graph 4).

Entire DC tissue exhibited PC-1 immunoreactivity. In DF, 7 cases (22.6%) exhibited PC-1 staining. In DC, mild PC-1 reactivity was seen in 5 cases (33.33%), moderate in 6 cases (40%) and intense in 4 cases (23.7%) along the basal cells. Suprabasal cells were not seen in 9 cases (60%) while mild reactivity was seen in 5 cases (33.3%) and moderate in 1 case (6.7%). The presence of epithelial stratification in 6 cases (60%) of DC and absent in 9 cases (75%) whereas 4 cases

(40%) shows stratification in DFs and 3 cases (25%) shows no stratification in DF as shown in Table 9, Graph 6.The difference was not statistically significant (P=0.384).

In all positive cases, PC-1 expression was observed in cytoplasm with varying degrees of intensity. In isolated foci in DF, few cells had exclusive cell membrane staining. In DC no such phenomenon was observed. The PC-1 staining was uniform in both DC and DF and was positive. In one case of DF odontogenic rests stained positive for PC-1 along with the epithelial layer. The stain appeared to be vesicular in nature and resembled that of liver sections. In cases where suprabasal layers staining were observed, staining was similar to that of basal layer. There was no difference in the intensity of staining in between layers in both DC and DF. Only little DF tissue was retrieved in three cases, as the study only included non-dilated, radiologically as well as histopathologically asymptomatic cases of impacted tooth. However in positive cases of DC, all the epithelial cells stained positive. In one DF case that was PC-1 positive, odontogenic cell rests also exhibited cytoplasmic PC-1 immunoreactivity. The staining was not diffuse like in epithelial cells but was granular in nature.

In the basal layer, mild staining was observed in 6 (27.3%), moderate in 9 (40.9%) and intense in 7 (31.8%) of all cases. Twelve cases had no suprabasal layers. Suprabasal cell layers expressed PC-1 in 10 cases of which 8 (80%) were mild and moderate staining in 2 cases (20%). On comparing the intensity of PC-1 staining, in basal cell layer, of all DC, 5 (33.33%) were mild, 6 (40%) was moderate and 4 (26.7%) was intense. In DF tissues, 1 (14.3%) was mild, 3 (42.9%) case each of moderate and intense. The difference in intensity of polcystin-1 staining between DC and DF was not statistically significant. (P = 0.595) (Table 11, Graph 8)

Of all PC-1 positive stained cases, only ten cases had suprabasal layer, of which 6 were DC and 4 were DF. Of these, 5 (33.3%) of DC and 3 (42.9%) of DF took mild staining and 1 (6.7%) of DC and 1 (14.3%) of DF took a moderate staining. This difference was not statistically significant. (P = 0.711) (Table 12, Graph 9)

The PC-1 staining intensity was compared among basal cells and suprabasal cells. Out of 22 cases of basal cell layer, 5 cases (33.3%) shows mild staining intensity in DC whereas 1 cases (14.1%) show mild staining in DF. 6 cases (40%) shows moderate staining intensity in DC whereas 3 cases (42.9%) shows moderate staining in DF. Four cases (26.7%) shows intense staining intensity in DC and 3 cases (42.9%) shows severe staining in DF. The difference was not significant. (P=0.595). Among 10 cases in suprabasal layer 5 cases (83.8%) shows mild staining intensity in DC whereas 3 cases (75%) shows mild staining in DF. One case (16.7%) show moderate staining in DC and 1 case (25%) shows moderate staining in DF. The difference was not significant (P=0.667). None of the suprabasal layer shows intense staining compare to basal layer as shown in table 13, graph 10.

In fully impacted teeth, the type of epithelium in DF was predominantly non-keratinized in 14 (60.9%) cases, para-keratinized in 6 (26.1%) cases. The difference was not statistically significant. (P=0.11) In maxillary third molar 7 (30.4%) cases are fully impacted and 1 (12.5%) case was partially impacted. In the mandibular third molar 16 (69.9%) cases are fully impacted and 6 (75%) cases are partially impacted. In canine 1 (12.5%) case was partially impacted. The difference was not statistically significant (P=0.16).

Clinical inflammation was present in 10 (43.5%) cases with fully impacted whereas 5 (62.5%) cases with partially impacted. The difference was not statistically significant (P=0.303). The duration of inflammation was chronic in 8 (34.8%) cases with fully impacted whereas 1 (12.5%) case with partial impaction. Mixed inflammation was seen in 7 (30.4%) cases with fully impacted whereas 2 (25%) cases with partially impacted. The difference was not statistically significant (P=0.339). The nature of the inflammation was diffuse in 6 (26.1%) with fully impaction. Diffuse inflammation was not seen in partial impaction. Localized inflammation was seen in 9 (39.1%) cases with fully impacted whereas in 3 (37.5%) cases with partial impaction. The difference was not statistically significant (P=0.204). (Table 3, Graph 3)

The difference in mean age, as measured by one-way ANOVA with regards to tooth type, gender, clinical inflammation, type of impaction and type of epithelium in DF are detailed in Table 4. None of these parameters was statistically significant. The mean age of DF tissues associated with maxillary 3rd molars was 26.38years whereas for mandibular 3rd molars it was 25.41years. The difference was not significant (P=0.876). The mean age of males presented was 24.67 years whereas mean age of was 25.91 years. This difference was not statistically significant (P=0.705). Those DF cases that presented with clinical inflammation, had a mean age of 23.87 years whereas those who are did not present with clinical inflammation was 27.13 years and the difference was not statistically significant (P=0.27). Those of the cases who presented with complete impaction had a mean age of 26.26 years while those with partial impaction had a mean age of 23.50 years. This difference was not statistically significant (P=0.415). The mean age of those DF cases that presented with para-

keratinized epithelium was 23.14 years, ciliated epithelium was 21 years, columnar epithelium was 29 years, non-keratinized was 26.32 and reduced enamel epithelium was 26.67 years. This difference was not statistically significant. (P=0.872) (Table 4)

The mean duration of clinical inflammation in males was 2.39 months whereas in females it was 3.35 months, the difference being not statistically significant (P=0.478). For the maxillary 3rd molar follicular tissue, the mean duration of clinical inflammation was 4.31 months whereas for mandibular molar follicular tissues it was 2.72 months. The difference was not statically significant. (P=0.438). Complete tooth impactions had mean duration of 3.59 months and while for partial impaction it was 1.59 months. The difference was not significant (P=0.152). Similarly the duration of clinical duration was not significant with respect to type of epithelium. (P=0.274) (Table 5).

The mean age of the DC patients was 36.80±15.02 years while in DFs 25.71±7.87 years. The difference was statistically significance P=0.002. When there was a presence of epithelial stratification, the mean age of the patient were 29.16±10.92 years whereas when it was absent it was 29.67±13.83 years. This difference was not statistically significant P=0.893. (Table 7)

The difference between the occurrence in DC and DF in terms of gender, epithelial stratification and PC-1 expression are described in Table 9, Graph 6. There was significant difference in terms of gender (P=0.010) and epithelial stratification (P=0.015). Among DC, 11 (73.3%) were males and 4 (26.7%) females while 10 (32.3%) males and 21 (67.7%) females had DF. Six cases(40%) of DC had

epithelial stratification, while it was observed in 25 cases (80.6%) of DF. (Table 8, Graph 5).

Among all the PC-1 stained cases the gender difference between DF and DC were studied. Among males 11 had DC (73.3%) while among DF 2 were males (28.6%). In DC 4 (26.7%) were females whereas 5 (71.4%) were females. The difference was statistically significant P=0.047. Among the PC-1 positive cases DC had the mean of 36.8±15.02 (95% CI 28.48 to 45.12) while DF had the mean 26.14±6.36 (95% CI 20.26 to 32.03) (P=0.089).The mean age of PC-1 positive cases was 33.41±13.70 years whereas for negative cases it was 25.58±8.37 years. The difference was statistically significant. (P=0.023) (Table 7).

Of all the 46 slides studied, PC-1 expression is observed in 22 instances (47.8%) and negative expression was seen in 24 cases (52.2%). Among those cases which has PC-1 expression 13 were males (59.1%) and 9 were females (40.9%). The difference between PC-1 positive and negative expression in terms of gender was not statistically significant (P=0.072). Epithelial stratification was absent in all negative cases, while present in 15 cases (68.2%) of all positive cases. While there was a presence of epithelial stratification only 7 cases answered for PC-1 as shown in table 12, Graph 9 and this difference was statistically significance after Yates correction.(P=0.000). The difference between the occurrence in DC and DF in terms of PC-1 expression was described in Table 10, Graph 7. There was statistically significant difference in PC-1 expression between DC and DF. PC-1 is positive in all DC while DF showed only 7 cases (22.6%) to be positive.(P =0.000).

Demographic parameters and frequency of oral lesions in patients with chronic renal diseases were also studied as a part of this study. Of 100 patients examined, 71 cases (71%) were males and 29 cases (29%) were females. The minimum age of the patients was 21 years and the maximum age was 70 years. The mean age was 43.06±11.98 years. Thirty six cases (36%) had type II diabetics, 35 cases (35%) had hypertension, 15 cases (15%) had renal stones, 86 cases (86%) were currently undergoing dialysis and 47 cases (47%) had renal failure. Five cases (5%) had undergone renal transplantation. Of these cases, 25% were categorized medically as end stage renal diseases while 7 cases had ADPKD and none of them were categorized as ESRD.(Table 14, Graph 11). The minimum blood urea levels in this population were 7.04 mg/dL and maximum level was 214.90 mg/dL. The mean urea level was 93.8±53.7 mg/dL. The minimum creatinine level was 1.48 mg/dL and the maximum was 71.3 mg/dL. The mean value was 9.23±8.78 mg/dL. The minimum hemoglobin percentage was 4.70% and maximum was 11.70%. The mean hemoglobin percentage was 8.09±1.84g/dL. The mean duration of treatment was 6.65 months.(0.5-60 months).

The minimum number of teeth present in the study population was 13 teeth and maximum full complement of teeth. The mean number of teeth was 29.78±3.08. No evidence of any cystic or impacted teeth was identified. The mean DMFT was 4.43±3.58.

The oral lesions present in the renal disease patients were as follows: 4 cases (4%) had oral candidiasis, 97 cases (97%) had chronic generalized gingivitis, 3 cases (3%) had generalized periodontitis, 8 cases (8%) had oral pigmentation, 3 cases (3%) had angular cheilitis, 1 case (1%) each of leukoplakia, oral submucous fibrosis, traumatic ulcer and uremic stomatitis. (Table 15, Graph 12).

Table 1

Demographic and Clinical features of dental follicles.

		Frequency	Percentage
Gender	Male	9	29.0
	Female	21	71.0
Teeth	Maxillary third molar	8	25.8
	Mandibular third molar	22	71.0
	Canine	1	3.2
Individual Teeth	Upper right third molar	3	9.7
	Upper left third molar	5	16.1
	Lower left third molar	9	29.0
	Lower right third molar	13	41.9
	Canine	1	3.2
Presence of clinical Inflammation		15	48.4
Type of impaction	Fully impacted	23	74.2
	Partially impacted	8	25.8

Graph 1

Demographic and Clinical features of dental follicles.

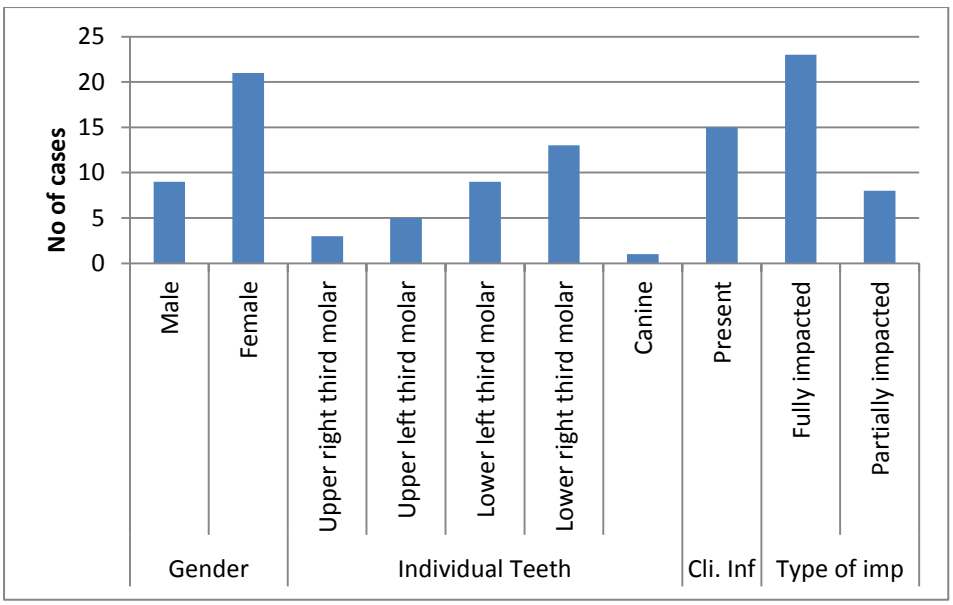


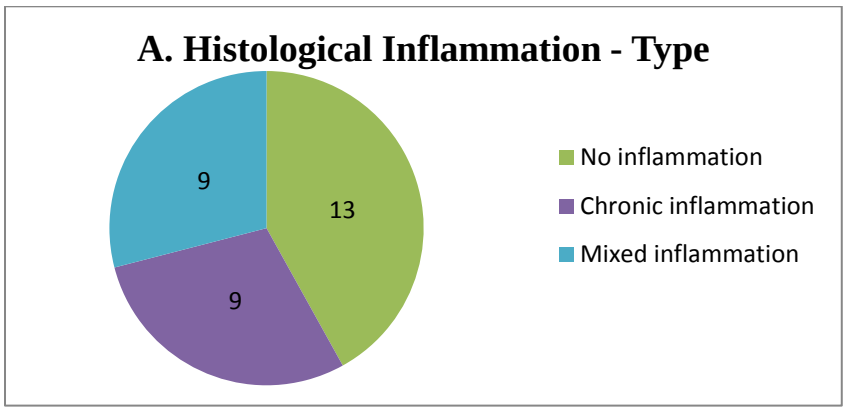
Table 2

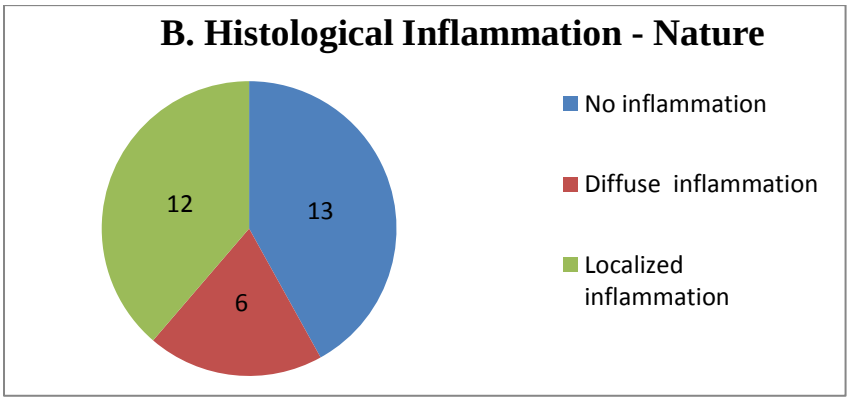
Histological and clinical features in dental follicles.

		Frequency	Percent
Histological inflammation - type	No inflammation	13	41.9
	Chronic inflammation	9	29.00
	Mixed inflammation	9	29.00
Histological inflammation - nature	No inflammation	13	41.9
	Diffuse inflammation	6	19.4
	Localized inflammation	12	38.7
Types of epithelium	Para-keratinized	7	22.6
	Ciliated	1	3.2
	Columnar	1	3.2
	Non –Keratinized	19	61.3
	Reduced enamel	3	9.7
Presence of odontogenic rests		4	12.9
Calcification	No calcification	23	74.2
	Eosinophilic	6	19.4
	Basophilic	2	6.5
Presence of vital bone		5	16.1
Connective tissue nature	Normal	3	9.7
	Fibrous	28	90.3
Presence of Giant cells		2	6.5

Graph 2

Histological and clinical features in dental follicles





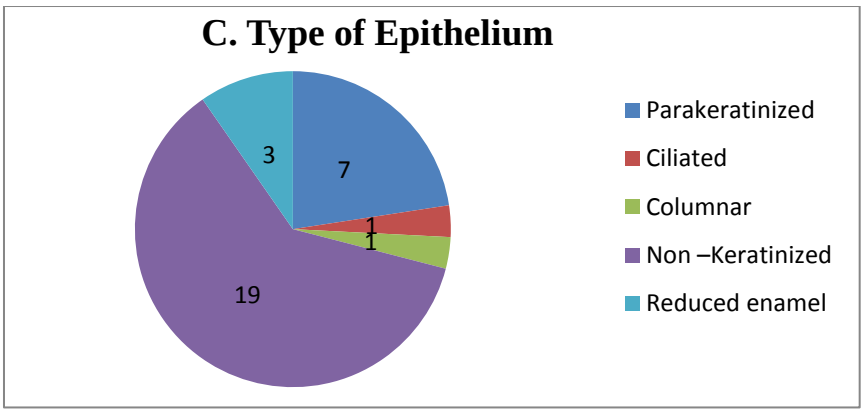


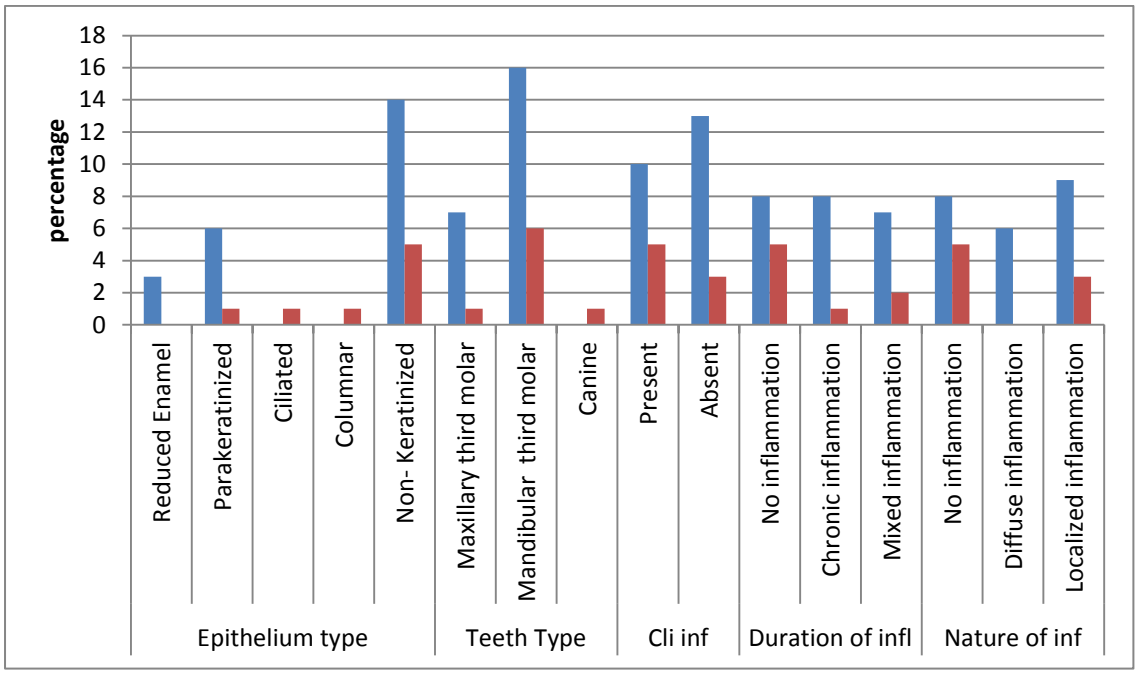
Table 3

Comparison of histological features in dental follicular tissue with grade of impaction

		Fully Impacted n=37 (%)	Partial Impaction n=8 (%)	P value
Epithelium type	Reduced Enamel	3(13)	-	0.112
	Para-keratinized	6(26.1)	1(12.5)	
	Ciliated	0	1(12.5)	
	Columnar	0	1(12.5)	
	Non- Keratinized	14(60.9)	5(62.5)	
Teeth Type	Maxillary third molar	7(30.4)	1(12.5)	0.162
	Mandibular third molar	16(69.9)	6(75)	
	Canine	0	1(12.5)	
Clinical Inflammation	Present	10(43.5)	5(62.5)	0.303
	Absent	13(56.5)	3(37.5)	
Duration of inflammation	No inflammation	8(34.8)	5(62.5)	0.339
	Chronic inflammation	8(34.8)	1(12.5)	
	Mixed inflammation	7(30.4)	2(25)	
Nature of inflammation	No inflammation	8(34.8)	5(62.5)	0.204
	Diffuse inflammation	6(26.1)	0	
	Localized inflammation	9(39.1)	3(37.5)	

Graph 3

Comparison of histological features in dental follicular tissue with grade of impaction



1. Fully Impacted 2.Partial Impaction

Table 4

Table comparing mean age of patients with clinical and histological features of dental follicles

		n	Mean	SD	95% CI for mean		P value
					Lower	Upper	
Tooth type	Maxillary 3rd molar	8	26.38	8.73	19.07	33.68	0.87
	Mandibular3rd molar	22	25.41	8.20	21.77	29.04	
	canine	1	22.00	-	-	-	
Gender	Male	9	24.67	6.12	19.96	29.37	0.70
	Female	22	25.91	8.87	21.97	29.84	
Clinical Inflammation	Present	15	23.87	5.63	20.75	26.98	0.27
	Absent	16	27.13	9.79	21.91	32.34	
Impaction	Complete	23	26.26	8.80	22.45	30.07	0.41
	Partial	8	23.50	5.53	18.88	28.12	
Epithelium	Parakeratinized	7	23.14	4.56	18.92	27.36	0.87
	Ciliated	1	21.00	-	-	-	
	Columnar	1	29.00	-	-	-	
	Non-Keratinized	19	26.32	9.40	22.58	28.52	
	Reduced enamel	3	26.67	8.96	4.4	48.93	

CI: Confidence Interval

Table 5

Table comparing the mean clinical duration of patients duration (in months) in dental follicles.

		n	Mean	SD	95% Cl for Mean		P Value
					Lower	Upper	
Gender	Male	9	2.39	2.44	.51	4.27	0.47
	Female	22	3.35	3.68	1.72	4.99	
Tooth type	Maxillary3rd molar	8	4.31	3.95	1.01	7.62	0.43
	Mandibular 3rd molar	22	2.72	3.16	1.31	4.12	
	Canine	1	1.00	-	-	-	
Impaction	Complete	23	3.59	3.68	2.00	5.18	0.15
	Partial	8	1.59	1.60	.26	2.93	
Epithelium	Parakeratinized	7	1.14	.84	.37	1.92	0.27
	Ciliated	1	.25	-	-	-	
	Columnar	1	.25	-	-	-	
	Non- keratinized	22	3.94	3.62	2.34	5.55	
	Reduced enamel	3	4	2	-0.97	8.97	

Table 6

Frequency of age, gender, nature, epithelial stratification and polycystin-1 expression.

		Frequency (n=46)	Percentage
Gender	Males	21	45.7
	Females	25	54.3
Nature	Dentigerous cyst	15	32.6
	Follicle	31	67.4
Epithelial stratification	Absent	15	32.6
	Present	31	67.4
Polycystin-1 expression	Negative	24	52.2
	Positive	22	47.8

Graph 4

Frequency of age, gender, nature, epithelial stratification and polycystin-1 expression.

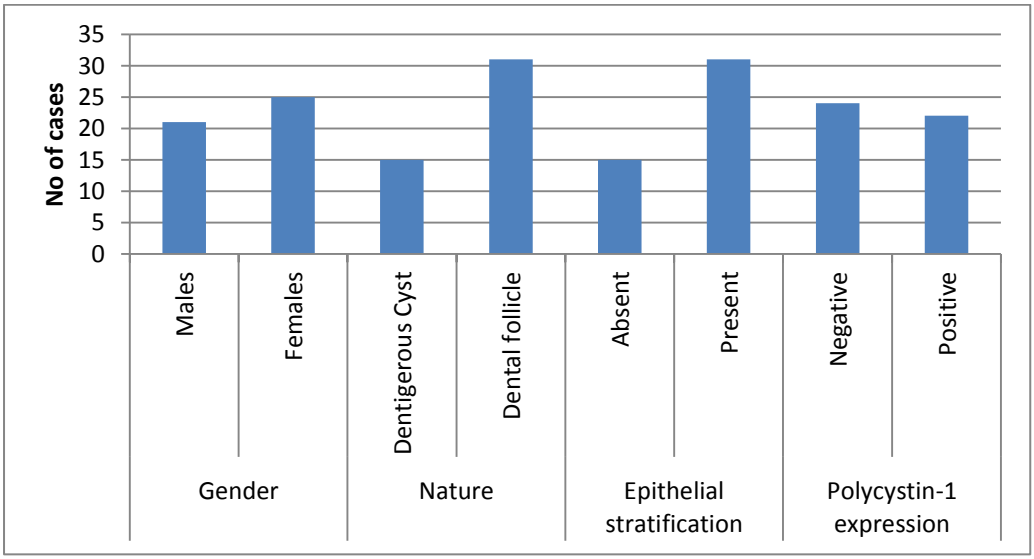


Table 7

Table comparing the mean age difference in Dentigerous cyst, Follicle, stratification and polycystin-1 expression.

Parameter		n	Mean	SD	95% CI of mean		P value
					Lower	Upper	
Nature	Dentigerous Cyst	15	36.8	15.0	28.4	45.1	0.002*
	Follicle	31	25.7	7.8	22.8	28.6	
Stratification	Absent	15	29.6	13.8	22.0	37.3	0.893
	Present	31	29.1	10.9	25.1	33.1	
Polycystin-1 reactivity	Negative	24	25.5	8.37	22.0	29.1	0.023**
	Positive	22	33.4	13.7	27.3	39.4	

***P value is lesser than 0.05 is statistically significant; CI: Confidence Interval**

Table 8

Analysis of gender, epithelial stratification and polycystin-1 expression between Dentigerous cyst (n=15) and Follicle (n=31).

		Dentigerous cyst n(%)	Dental follicle n(%)	P value
Gender	Males	11(73.3)	10(32.2)	0.010*
	Females	4(26.7)	21(67.7)	
Epithelial stratification	Absent	9(60)	6(19.4)	0.015
	Present	6(40)	25(80.6)	
Polycystin-1 expression	Negative	0(0)	24(77.4)	0.000*
	Positive	15(100)	7(22.6)	

***P value is lesser than 0.05 is statistically significant;**

Graph 5

Analysis of gender, epithelial stratification and polycystin-1 expression between dentigerous cyst (n=15) and Dental follicle (n=31).

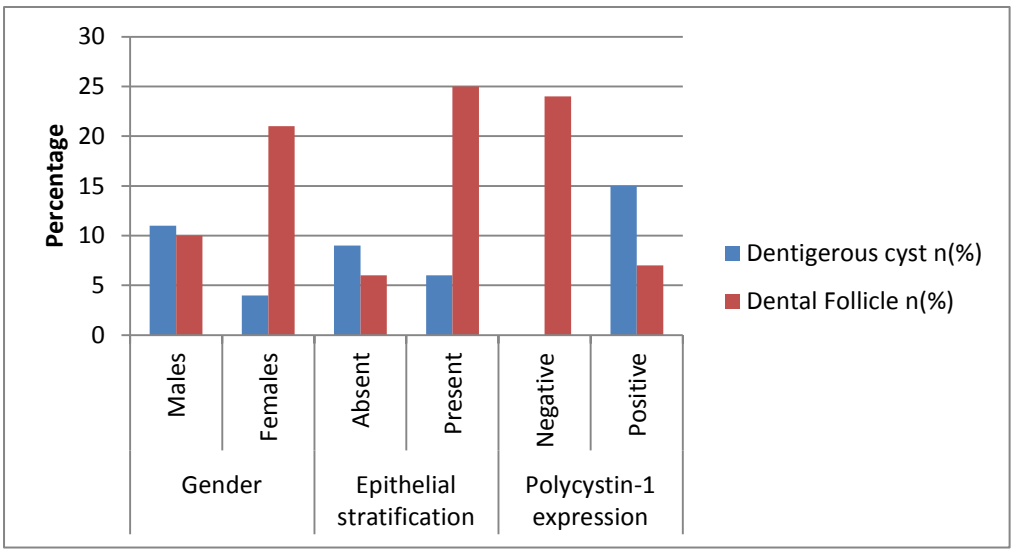


Table 9

Analysis of epithelial stratification in Dentigerous cyst (n=15) and Dental follicle (n=31).

	Epithelial stratification		P value
	Absent(n=12) n%	Present(n=10) n%	
Dentigerous cyst	9(75)	6(60)	0.384
Dental follicle	3(25)	4(40)	

Graph 6

Analysis of epithelial stratification in Dentigerous cyst (n=15) and Dental follicle (n=31).

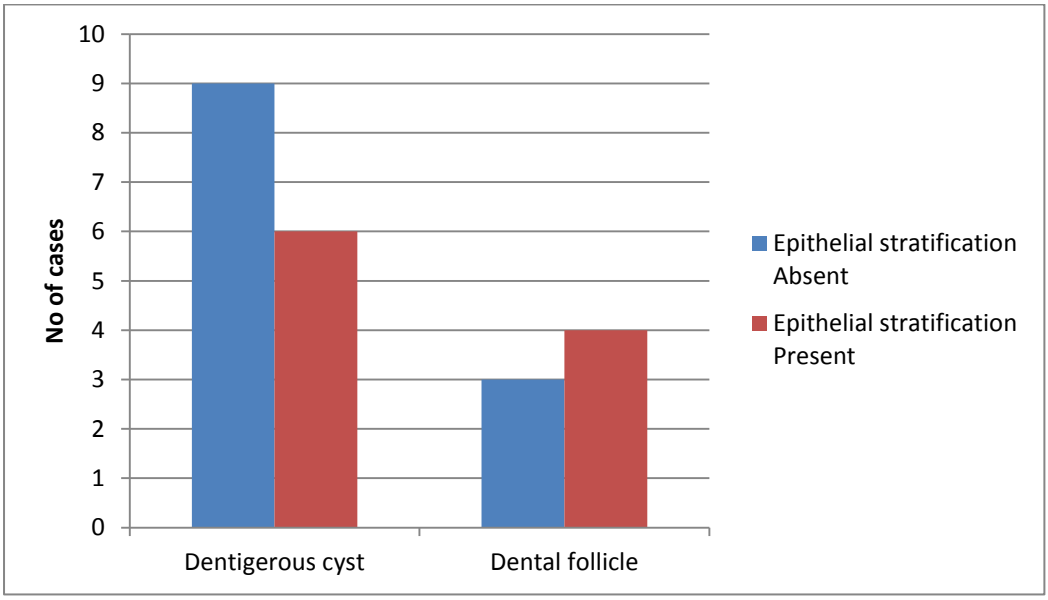


Table 10

Analysis of gender and epithelial stratification in Polycystin-1 expression in basal cells

		Polycystin-1 Expression in basal cells		P value
		Negative n(%)	Positive n (%)	
Gender	Males	8 (33.3)	13 (59.1)	0.072
	Females	16 (66.7)	9 (40.9)	
Epithelial stratification	Absent	0	15(68.2)	0.000*
	Present	24(100)	7 (31.8)	

**P value is lesser than 0.05 is statistically significant

Graph 7

Analysis of gender and epithelial stratification in Polycystin-1 expression in basal cells

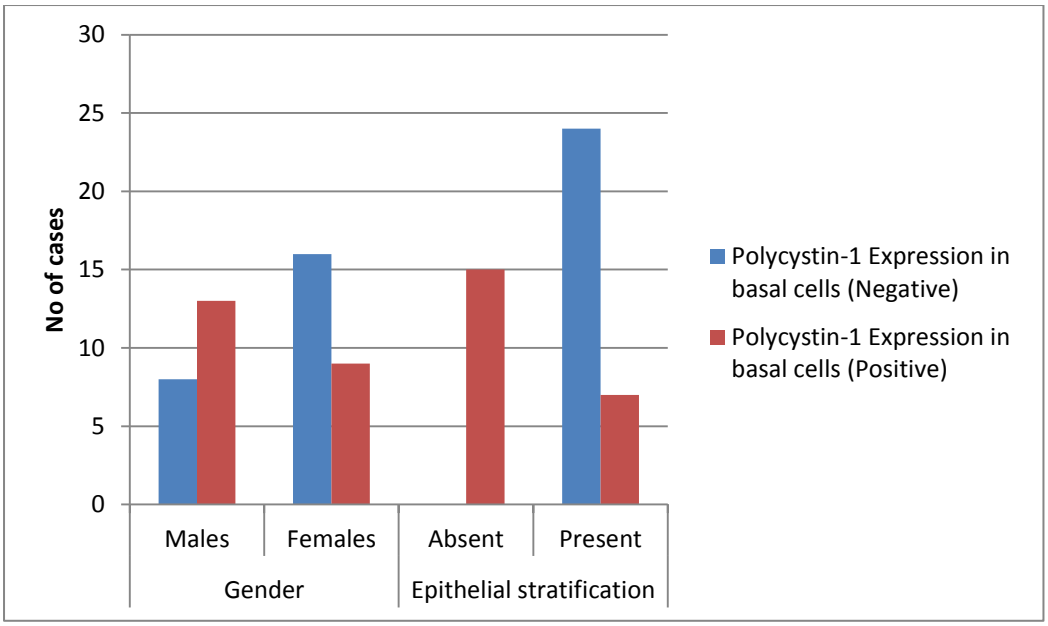


Table 11

Table comparing the intensity of polycystin-1 expression in basal layer between dentigerous cyst (n=15) and Dental follicle (n=31)

	Dentigerous cyst (n=15)	Dental follicle (n=31)	P Value
Mild	5(33.3%)	1(14.3%)	0.59
Moderate	6(40%)	3(42.9%)	
Intense	4(26.7%)	3(42.9%)	

Graph 8

Graph comparing the intensity of polycystin-1 expression in basal layer between dentigerous cyst (n=15) and Dental follicle (n=31)

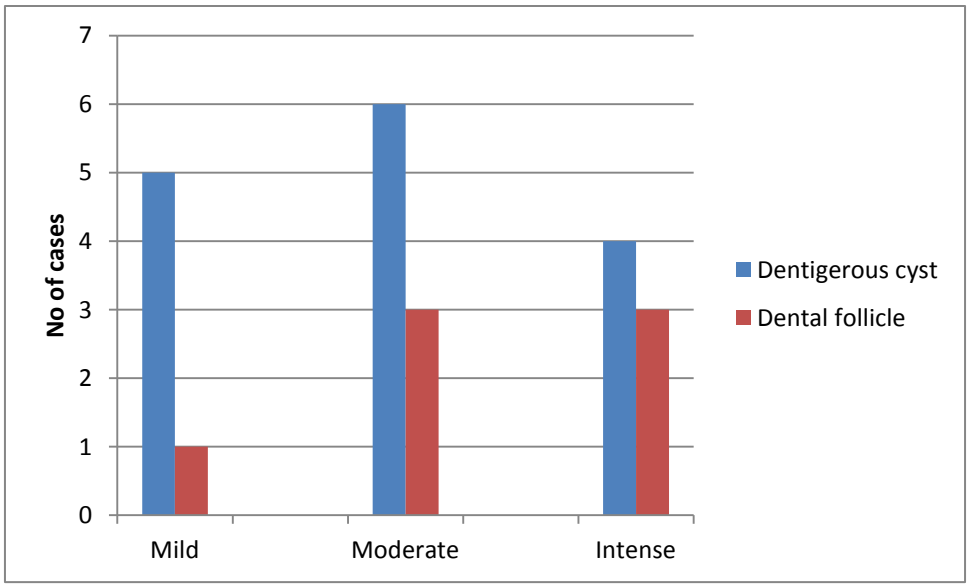


Table 12

Table comparing the intensity of polycystin-1 expression in suprabasal layer between Dentigerous cyst (n=15) and Dental follicle (n=31)

	Dentigerous cyst (n=15)	Dental follicle (n=31)	P Value
Absent	9(60%)	3(42.9%)	0.711
Mild	5(33.3%)	3(42.9%)	
Moderate	1(6.7%)	1(14.3%)	

Graph 9

Graph comparing the intensity of polycystin-1 expression in Suprabasal layer between dentigerous cyst (n=15) and Dental follicle (n=31).

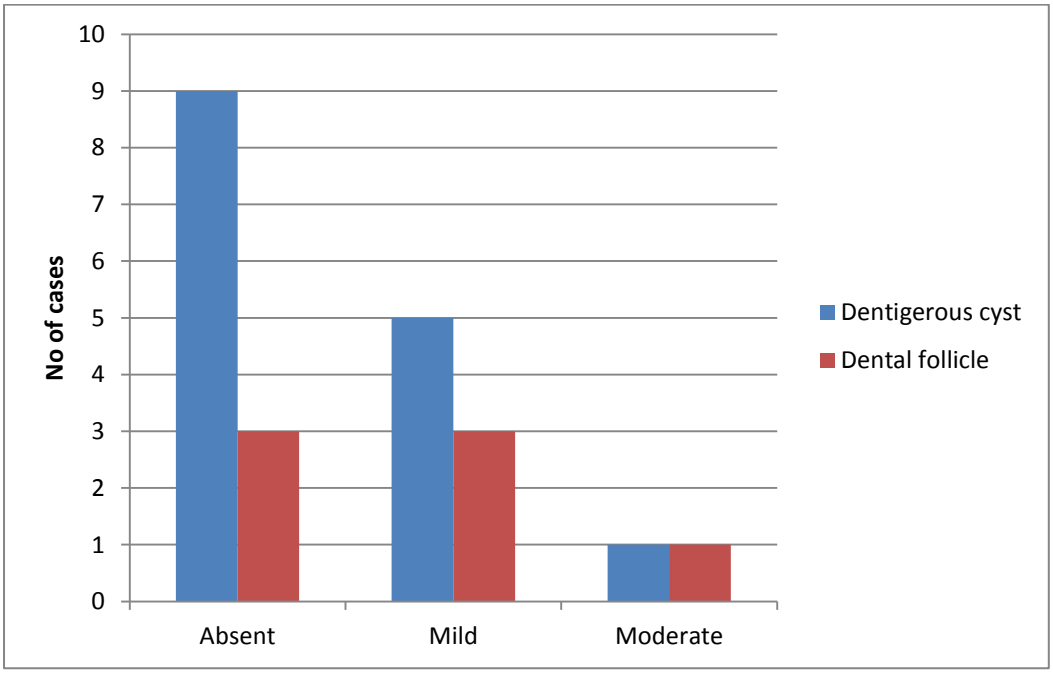


Table 13

Table comparison of basal and suprabasal layer staining intensity with Polycystin-1 in dentigerous cyst (n=15) and Dental follicle (n=31).

Polycystin-1 staining intensity		Dentigerous Cyst n(%)	Dental follicle n(%)	P Value
Basal cell layer (n=22)	Mild	5(33.3)	1(14.3)	0.595
	Moderate	6(40)	3(42.9)	
	Intense	4(26.7)	3(42.9)	
Suprabasal cell layer (n=10)	Mild	5(83.8)	3(75)	0.667
	Moderate	1(16.7)	1(25)	

Graph 10

Graph comparison of basal and suprabasal layer staining intensity with Polycystin-1 in dentigerous cyst (n=15) and Dental follicle (n=31).

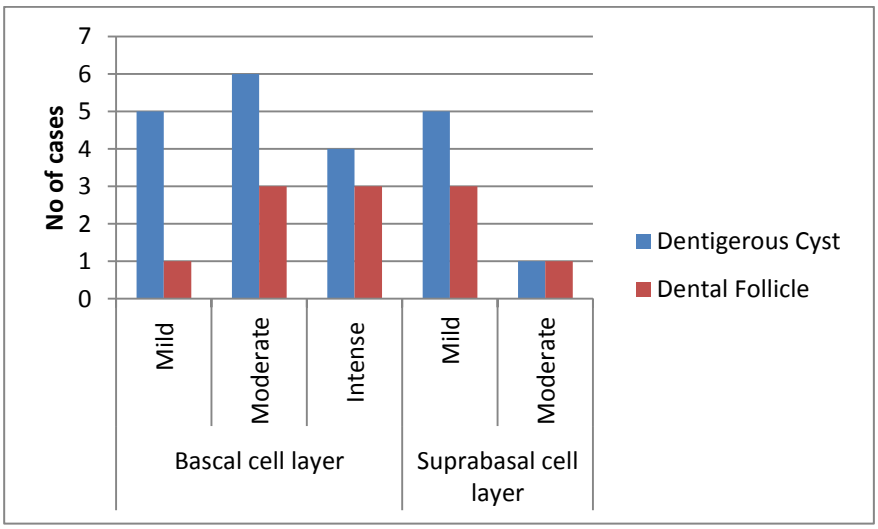


Table14

Analysis of gender, systemic disease and type of renal diseases in 100 chronic kidney disease patients.

		Frequency	Percentage (%)
Gender	Male	71.0	71.0
	Female	29.0	29.0
Systemic diseases	Type II Diabetics	36	36.0
	Hypertensive	35	35.0
Renal diseases	Renal Stone	15	15.0
	Dialysis	86	86.0
	Renal failure	47	47.0
	Renal Transplantation	5	5.0
	End stage renal disease	25	25.0
	Polycystic kidney disease	4	4.0

Graph 11

Analysis of gender, systemic disease and type renal diseases in 100 Chronic Kidney Disease patients.

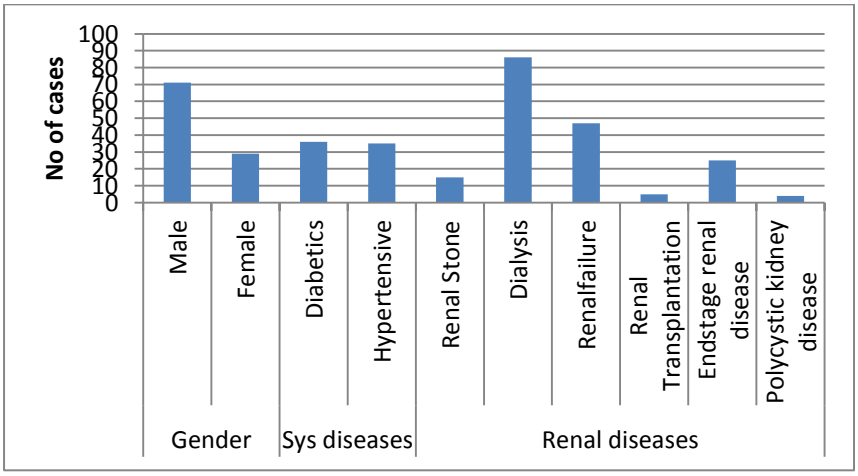


Table 15

Frequency of oral lesions in chronic renal disease patients (n =100)

Oral Lesions	Frequency	Percentage (%)
Oral Candidiasis	4	4.0
Chronic Generalised Gingivitis	97	97.0
Generalised Periodontitis	3	3.0
Pigmentation	8	8.0
Angular Cheilitis	3	3.0
Leukoplakia	1	1.0
Oral Submucous Fibrosis	1	1.0
Traumatic Ulcer	1	1.0
Uremic Stomatitis	1	1.0

Graph 12

Analysis of oral lesions in chronic renal disease patients

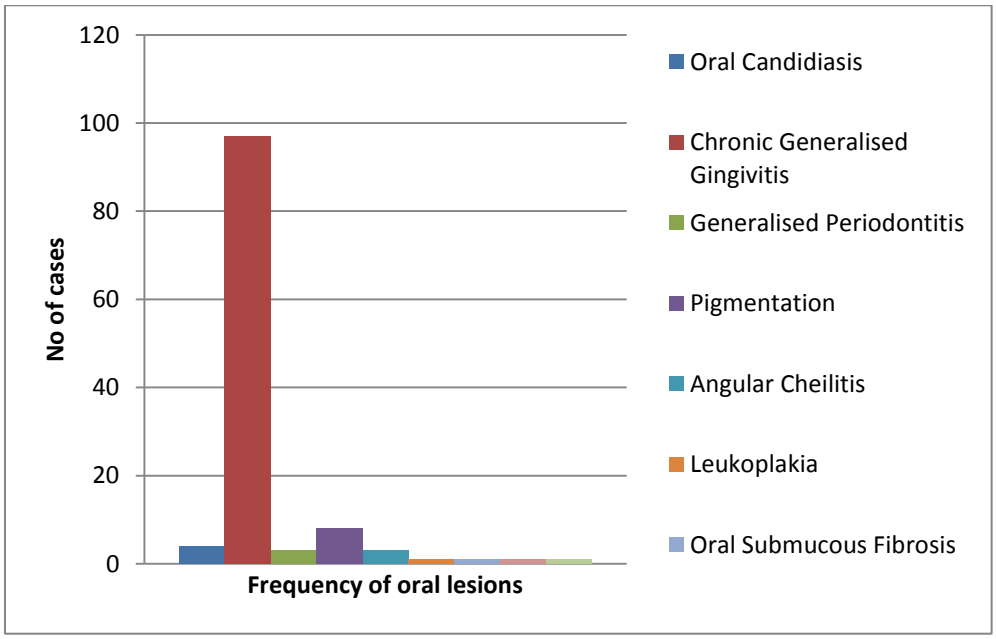


Figure 1: Armamentarium

Figure 2: Primary Antibody

Figure 3: Secondary Antibody

Figure 4: Photomicrograph showing Non-Keratinized Stratified Squamous Epithelium in clinical / radiographically asymptomatic Dental follicle

H&E 10X View

H&E 40 X View

Figure 5: Photomicrograph showing Reduced Enamel Epithelium in clinical / radiographically asymptomatic Dental follicles

H&E 10X View

H&E 40X View

Figure 6: Photomicrograph showing Para-Keratinized Stratified Squamous Epithelium in clinical / radiographically asymptomatic Dental follicles

H&E 10X View

H&E 40X View

Figure 7: Photomicrograph showing Odontogenic rests in clinical / radiographically asymptomatic Dental follicles

H&E 10X View

H&E 40X View

Figure 8: Photomicrograph showing Polycystin-1 positive of Liver (Control Tissue)

PC-1 (IHC) 10X View

PC-1 (IHC) 40X View

Figure 9: Photomicrograph showing Polycystin-1 positive cells (Dentigerous cyst)

PC-1 (IHC) 10X View

PC-1 (IHC) 40X View

Figure 10: Photomicrograph showing Polycystin-1 positive cells (Dental Follicle)

PC-1 (IHC) 10X View

PC-1 (IHC) 40X View

Figure 11: Photomicrograph showing Polycystin-1 positive of cells

(Dental Follicle)

PC-1 (IHC) 10X View

PC-1 (IHC) 40X View

Figure 12: Photomicrograph showing Polycystin-1 positive in Odontogenic Rests (Dental Follicle)

PC-1 (IHC) 10X View

PC-1 (IHC) 40X View

The present study was done to ascertain the association of an integral transmembrane protein, Polycystin-1 (PC-1) in cystogenesis of DC cyst from DF tissues. PC-1 is known to play a vital role in cystogenesis of renal and extra-renal ADPKD cysts.[9]

Only inflammatory, infective and immunological related lesions such as oral candidiasis, dental caries, gingivitis and periodontitis were common in the chronic renal disease patients. CRD associated lesions such as uremic stomatitis or uremic breath was uncommon.

DF tissues are reported to be histologically characterised by variation in lining epithelium, inflammatory changes and calcification. In the present study, the frequency of parakeratinised stratified squamous epithelium was observed in 7 cases (22.6%), ciliated epithelium was seen in 1 case (3.2%), columnar epithelium was seen in 1 case (3.2%), non-keratinised stratified squamous epithelium was seen in 19 cases (61.3%) and REE was seen in 3 cases (9.7%). Our finding concurs with earlier reports in recent literature. **Kotrashetti VS et al.,**[13] reported that of all DF tissues examined, 36.5% show REE, 51.2% show stratified squamous epithelium and 12.19% had no epithelium. **Kim J**[51] found that 54% of cases of his study show REE.[51] **Mesgarzadeh AH et al.,**[52] observed 3% of his cases to have non-keratinised stratified squamous epithelium. **Yadav M et al.,**[12] found 16.21% REE and 16.21% show hyperplastic epithelium. **Saravana GH et al.,** [4] found 46% of DF tissue to have stratified squamous epithelium. The minor variation in the type of epithelium could be accounted to age of patients and inflammation.[23, 24, 51]

In the present study chronic inflammatory cells were observed in 9 cases (29%) of DF and mixed inflammatory cells in 13 cases (41.9%) of DF. **Kotrashetti VS et al.,**[13] reported that 10 DF show inflammatory component in the stroma and **Yadav M et al.,**[12]

found chronic inflammation which was seen in 75.55% of cases. The difference could originate on the duration of clinical inflammation, timing of removal of tooth as well as the surgical modality by which the tooth was disimpacted.[13]

Present study identified calcification in 8 cases of which 6 cases (19.4%) stained eosinophilic and 2 cases (6.5%) basophilic in nature. **Kotrashetti VS et al.,**[13] reported 13 DF show dystrophic calcification. **Kim J et al.,** [51] found that foci of calcification were seen in 37% of their cases. This variation of occurrence would be explained by the age factor and patient population as well as degree of inflammatiom.[51]

The histopathological findings of DF tissues with epithelium from clinical and radiologically asymptomatic impacted teeth revealed no abnormalities. None of the DF tissue sections exhibited features suggestive of any other odontogenic or non-odontogenic pathology. On the contrary, several authors have identified several degrees of pathology in DF tissues. Several authors have reported varying percentage of pathoses in association with DF tissues around impacted teeth. They include **Kim Jet al.,** [51] 1993 (53%), **Hussain T et al.,**[14] 2005 (1.73%), **Saravana GH et al.,**[4] 2008 (46%), **Musgarzadeh AH et al.,**[52] (53%), **Yadav M et al.,**[12] 2011 (4.44%) and **Kotrashetti VS et al.,**[13] 2012 (58.5%). The reported range of prevalence of pathoses in asymptomatic DF tissue is in between 1.73% to 53%. The difference is related to the population, study criteria and definitions employed in the study. In the present study, as DF tissues were isolated from a small number of clinically and radiologically asymptomatic impacted teeth, the incidence of abnormalities is nil.

The clinical and demographical features of DC observed in the present study concur with those reported in literature.[22, 23, 24, 51] DC and DF tissue differs each other in clinical,

radiological and histopathological features. The difference between two of these entities have been always a diagnostic dilemma especially when the radiolucent lesion associated with impacted teeth are small. The radiological size of the DF has often used to define the entity. When the space between the tooth and the tissue is more than 2.5 mm, it is usually accepted to pathological while those below 2.5 mm have been considered to be dilated dental follicle. It has been reported that DC was the most common misdiagnosis of dental follicles.[51] Normal DF, the epithelium is often fragmented or separated. This is probably because there is a stronger attachment of the epithelium to enamel surface of the tooth than to the underlying DF.[51] Though there are many explanations accounting for the mechanism of transition from follicle to DC, literature has not identified a single most important factor or feature that distinguishes the follicle from DC. Several epidemiological studies have identified thickness of the pericoronal radiolucency to be a reliable marker for differentiating DC from normal structures.[2]

Abnormal subcellular localization of PC-1 has been associated with 86% of ADPKD and often co-localizes with Polycystin-2.[9] The monoclonal antibody against LRR region [epitope-7e12] near the N terminal of the PC-1 structure. Certain immunohistochemical studies with PC-1 have identified difficulties due to antibody specificity and low levels of expression in cells and tissues. Few PC-1 N terminal antibodies have been demonstrated to be sensitive but lack specificity as they stain fraction or a truncated part of the PC-1.[37] Previous immunohistochemical studies have confirmed that the antibody raised against this epitope has been PC-1 specific and has demonstrated the immunoreactivity on vast majority of ADPKD cysts.[34] Subcellular localization of PC-1 is mainly on cell surface membrane and may be changed in certain condition to show a cytoplasmic staining pattern as in 86% of ADPKD cysts.[37] Normal PC-1 localization in

cytoplasm could be due to its transportation as a precursor protein or unknown or hitherto undescribed function in the cytoplasm as many normal cells and tissues carry this protein in a cytoplasmic location. PC-1 is expressed in human cells and tissues involved in functions such as secretion or transport of molecules, highly organised cells with selected membrane domains involved in specific functions. Cells that are subjected to great mechanical pressure, one that requires a high number of adhesion molecules stain more intensively for PC-1. Often a high number of membrane interactions between cells often correlate with high immunoreactivity of PC-1.[38]

The PC-1 immunoreactivity of various odontogenic tissues has not been described in literature. To best of our knowledge, this study is the first of its kind to study the PC-1 immunoreactivity in odontogenic tissues. Previous studies have analysed and reported the pattern of PC-1 immunoreactivity in skin and salivary gland only.[38]

A proportion of ADPKD cysts (about 20%) did not reveal immunoreactivity with PC-1 antibody raised against N terminal epitope, indicating that these mutated protein could be rendered unstable when truncated as reported by **Ong ACM**.[34] In the present study, all DC epithelial cells stained for PC-1 with different staining intensity along the entire cytoplasm where as 22.6% of DF epithelial tissues stained positive for PC-1. In such sections, there had been few areas in DF section where cell membrane stained for PC-1 while in certain areas it did not react indicating a possible absence of expression. This could also be either due to the unstable nature of mutated protein or absence of the expression of a PC-1 in a clone of cell of population, indicating a polyclonal proliferation of cells in DF epithelial tissues.[37]

The staining pattern of PC-1 in ADPKD renal and extra-renal cysts has been reported. In case of ADPKD, the stain is taken by the cytoplasm while in normal instances the staining pattern is negative to cell membrane localization depending upon the cellular location in kidneys, stage of development and variation of antibodies used for the study.[34, 38, 39, 52] In the present study, though variation in terms of staining intensity has been observed in DC, all epithelial cells had positive immunoreactivity for PC-1. The staining was diffuse and uniform in all neighbouring cells. None of the DC had exclusive membrane staining alone. Similar reactivity has been reported in renal cysts associated with ADPKD.[34,36 ,38,39,53] In contrast, only 22.6% of DF epithelium stained positive for PC-1 and this staining pattern was also different in DC.

In 22.6% cases of DF tissue, epithelium exhibited PC-1 cytoplasmic immunoreactivity and the difference of PC-1 immunoreactivity between DC and DF tissues was statistically significant (P = 0.000). Abnormal cytoplasmic expression of PC-1 in all DC epithelium indicates that the PC-1 is associated with cystic transformation. Epithelial stratification (Table 9, P = 0.384) did not appear to influence PC-1 expression whereas there was a significant difference between DC and DF (Table 9, P = 0.015). Gender (Table 8, P = 0.01) and mean age (Table 7, P = 0.002) was significantly different between DF and DC. PC-1 immunoreactivity difference among gender (Table 10, P = 0.000) and mean age (Table 7, P = 0.023) was significant. These results indicate that gender, age and stratification did not influence the PC-1 expression in between DC and DF. The PC-1 immunorectivity in DF epithelium widely varied. In certain foci, epithelial cells did not take up the stains while in certain focus, only membrane staining was observed. The results of table 9-12 indicate that polycystin-1 immunoreactivity is related to DC cystogenesis and is independent of age, gender and stratification. These results indicate that the PC-1 positive

DF tissues are probably exhibiting changes that could progress to DC on alonger timescale through the proliferation of a monoclonal set of cells in which PC-1 gene is mutated.[45]

The intensity of PC-1 immunoreactivity between DC and positive DFepithelial tissue at the basal cell level (P= 0.595) and suprabasal level (P = 0.711) did not differ significantly. The variation in between the basal and suprabasal cell layers in all PC-1 positive sections was also not significant. This indicates that there is no difference in expression between layers of epithelium both in DC and DF tissues, when there is PC-1 expression. As the DF tissue is exposed to same mechanical stresses similar to DC, in all layers, expression of PC-1 in DC could not be attributed to mechanical stress as sited in literature.[38]

Intracellular localization of PC-1 has been associated with a membrane like structure when microscope reveals a diffuse staining pattern in Immunohistochemistry.[52] In the present study, odontogenic rests in PC-1 positive DF tissues show granular structures similar to that of control liver sections whereas odontogenic rests in PC-1 negative DF epithelial tissue does not exhibit this staining. This indicates that there is a gross, abnormal accumulation of PC-1 at the odontogenic tissue level. More ever recent works have postulated a dose dependent relationship of PC-1 with the severity of disease.[48] Exosomes has demonstrated to be linked to PC-1 expression.[48] Ameloblasts also have similar multivesicular bodies.[19] These observations indicate that there is a high probability that PC-1 could be accumulated in DF epithelium and produce conditions that directs cystogenesis.

Loss of localization in cell membrane and cytoplasmic accumulation has been associated with ADPKD.[39] Mutations that prevent PC-1's GPS cleavage, an early event in

secretory pathway, also prevent its plasma membrane leading to cystogenesis. Probably low level PC-1 mutations (spontaneous or acquired) manifesting during late odontogenesis, could lead to abnormal subcellular localization and quantity carry difference in PC-1 immunoreactivity between established DC and apparently normal DF tissues.

In experimental cell culture studies, PC-1 knockdown lead to haplo insufficiency that subsequently triggered proliferation, increased adhesion to collagen type I and increased apoptosis. It had been demonstrated that such down regulation of PC-1 in its functional position leads to increased expression of $\alpha_2\beta_1$ integrin in cell surface and reduced apoptosis.[41]DC has been demonstrated to have increased expression of $\alpha_2\beta_1$, particularly in basal cell layer as compared to DF epithelium.[26] The integrin α_2 subunit has been associated with stratification and its down regulation is associated with decrease in cell-cell adhesion and predisposes desquamation of superficial cells, in PC-1 positive DC and DF cells, probably abnormal PC-1 content increases $\alpha_2\beta_1$ integrin that cause strong attachment of basal cells to the connective tissue and this phenomenon may be PC-1 dose dependent. In MDCK cells, co-localization of PC-1 with desmoplakin (I, II) proteins in the cytoplasmic part of desmosomal plaque only has been observed. (Absent in tight or adherens junctions). Through all this mechanism abnormal PC-1 probably interferes with normal signal transduction, cell-cell adhesion, protein sorting and cell polarity which contribute to cystogenesis.[44] The same mechanism could also occur in DC.

PC-1 has been associated with primary cilium and is regarded as a major ciliary protein.[43,45] Identification of a primary cilium in DC in a patient with ADPKD has led to hypothesis that DC cystogenesis is mediated by primary cilium.[5]A defect in primary cilium function contributes to pathogenesis of PKD. Loss of PC-1 causes loss of ciliary function. PC complex act as a receptor and perhaps functions as a mechanosensor signalling Ca^{2+}

influx through a TRP channel after movement detection. PC-1 coated ELV mediate signalling at cilium and probably interferes with intracellular cAMP.[54] Alteration in intracellular cAMP has also been hypothesized as a mechanism for DC cystogenesis.[5] Abnormality of Shh, Ptch along with Wnt pathway have been associated with DC cystogenesis.[5] All these pathways have been noted to be mediated through primary cilium which in turn associated with Polycystin-1.[45,54]

The findings of our study indicate a possible role of PC-1 in the pathogenesis of Dentigerous cyst, which need further evaluation by larger studies and using molecular techniques.

- The aim of the present study was to evaluate and compare the immunoreactivity of Polycystin-1 in clinically, radiologically and histopathologically confirmed cases of dentigerous cyst and dental follicles and to estimate the prevalence of dentigerous cyst in chronic renal disease patients.
- It was observed that no clinical evidence of dentigerous cyst was present in 100 chronic renal disease patients
- Totally 15 cases of dentigerous cyst and 31 follicles were taken for immunohistochemical study.
- All dentigerous cysts were positive for polycystin-1 and 7 out of 31cases (22.6%) of asymptomatic follicles were positive for polycystin-1.
- The Polycystin-1 in dentigerous cyst was entirely epithelium and cytoplasmic.
- In addition to cytoplasmic staining some cases of follicles exhibited isolated foci of patchy epithelial membranous staining.
- The Polycystin-1 expression between dentigerous cyst and follicles were statistically significant (P = 0.000). The intensity of staining increased between the epithelial cells of dentigerous cyst and follicles.
- The significant difference in the expression of Polycystin-1 between dentigerous cyst and follicle suggests that Polycystin-1 may have a role to play in the transformation of follicle to dentigerous cyst.

1. Contribution to the diagnosis of the small dentigerous cyst.

   Damante JH, Fluery RN

   Pesquisa Odontologica Basileira 2001; 15: 238-46.

2. Pericoronal follicles of asymptomatic impacted teeth: A radiographic, histomorphologic, and immunohistochemical study.

   Villalba L, Stolbizer F, Blasco F, Maurino NR, Piloni MJ, Keszler A.

   International Journal of Dentistry 2012; 935310:1-6.

3. Epidermal growth factor receptor distribution in pericoronalfollicles: relationship with the origin of odontogenic cysts and tumors.

   Baumgart CS, Lauxen IS, Filho MSA, Quadros OF.

   Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, Endodontics 2007; 103: 240-45.

4. Cystic changes in dental follicles associated with radiographically normal impacted mandibular third molar.

   Saravana GHL, Subharaj K

   British Journal of Oral and Maxillofacial Surgery 2008; 46: 552-53

5. Primary cilia in the pathogenesis of dentigerous cyst: a new hypothesis based on role of primary cilia in autosomal dominant polycystic kidney disease.

   Anoop UR, Verma K, Narayanan K.

   Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, Endodontics 2011; 111(5): 608-17.

6. Oral manifestations of renal patients before and after transplantation: A review of literature;

   Mozaffari PM, Amirchaghmaghi M, Mortazavi H.

Dental Journal of Hamadan 2009; 1: 1-6.

7. Dental considerations for the patient with renal disease.

   ÁlamoSM, EsteveCG , Perez MGS.

   Journal of Clinical Experimental Dentistry 2011; 3(2):112-9.

8. End-stage renal disease in india and pakistan: incidence, causes and management.

   Sakhuja V, Kohli HS.

   Ethnicity and Diseases 2006;16 :21-23.

9. Polycystin: new aspects of structure, function, and regulation.

   Wilson PD.

   Journal of American Society of Nephrology 2001;12: 834–45.

10. Autosomal dominant polycystic kidney disease: pathophysiology and treatment.

    Rapoport J.

    Quality Journal of Medicine 2007; 100:1–9.

11. Polycystin: *In vitro* synthesis, *in vivo* tissue expression and subcellular localization identifies a large membrane-associated protein.

    Beskrovnaya OI, Dackowski WR, Foggensteiner L, Coleman N, Thiru S, Petry LR, Burn TC, Connors TD, Raay TV, Bradley J, Qian F, Onuchic LF, Watnick TJ, Piontek K, Hakim RM, Landes GM, Germino GG, Sandford R, Klinger KW.

    Proceedings of the National Academy of Sciences 1997; 94: 6397-02.

12. The wisdom behind third molar extraction, A clinicopathologic study.

Yadav M, Meghana SM, Deshmukh A, Godge P.

International Journal of Oral Pathology 2011; 2(3):7-12.

13. Histopathologic changes in soft tissue associated with radiologically normal impacted third molars.

Kotrashetti VS, Kale AD, Bhalaerao SS, Halikeremath SR.

Indian Journal of Dental Research 2010; 21(3): 385-90.

14. Pathology associated with impacted mandibular molars in a group of Jordanians.

Hussiain TK, Bataineh AB

Journal of American Association of Oral and Maxillofacial Surgeons 2005; 11: 1598-01.

15. Correlation of the radiological and morphological features of dental follicles of third molars with incomplete root formation.

Oliveira DM, Andrade ESS, Silveira MMF, Camargo IG.

International Journal of Medical Sciences 2008; 5:36-40.

16. Histopathologic evaluation of follicular tissues associated with impacted third molars.

Khorasani M, Samiezadeh F.

Journal of Dentistry 2008;5: 65-70

17. Pathologically significant pericoronal lesions in adult: Histopathologic evalution.

Curran AE, Damm DD, Drummond JF.

Journal of Oral and Maxillofacial Surgery 2002; 60; 613-17.

18. Phase-contrast and electron microscopic study of the junction between reduced enamel epithelium and enamel in unerupted human teeth.

Listgarten MA.

Archives of Oral Biology 1966;11:999-16.

19. Application of high-resolution immunocytochemistry to thestudy of the secretory, resorptive, and degradative functions of ameloblasts.

Nanci A, Slavkin HC, Smith CE.

Advanced Dental Research 1987;1:148-61.

20. Cysts of the oral and maxillofacial regions, 4th Edition.

Shear M and Speight PM. (Eds)

Blackwell Publishing Asia Private Ltd; Australia. Page 59-75.

21. A review of recent experiment work on the dental cyst.

Haris MH.

Proceedings of the Royal Society of Medicine 1974; 67: 1259-62

22. The Small Dentigerous cyst: A diagnostic dilemma.

Daley TD, Wysocki GP

Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, Endodontics 1995; 79: 77-81.

23. A Comparative histopathological study of epithelial linings of odontogenic cysts and unicystic ameloblastomas.

Sudiono J, Zain RB

Annals of Dentistry 1998; 5: 29-34.

24. Clinicipathological study of dentigerous cysts in Singapore and Malaysia.

Yeo JF, Zain RB, Lian ST, Zhao YY, Ngeow WC.

Malaysian Journal of Pathology 2007; 29: 41-47.

25. Expression of basement components in odontogenic cysts.

    Poomsawat S, Punyasingh J, Weerapradist W.

    Oral Diseases 2006; 12: 290-96.

26. Immunohistochemical profile of integrins in enlarged dental follicles and dentigerous cysts.

    Godoy GP, Silveira JD, Lins RDAU, Souza LB, Freitas RA, Queiroz LMG.

    Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, Endodontics 2007; 104: 29-34.

27. Odontogenic Cysts and Tumors In Oral and Maxillofacial Pathology, 3rd Edition, Elsevier- Reed, Gurgaon, Page 700.

    Neville BW, Damm DD, Allen CM, Bouquot JE. (Eds)

28. Uremic stomatitis in chronic renal failure.

    Leao J C, Gueiros LAM, Segundo AVL, Carvalho AAT, Barrett W, Porter SR.

    Journal of Clinics 2005;60(3):259-62.

29. Oral and Dental Aspects of Chronic Renal Failure.

    Proctor R, Kumar K, Stein A, Moles D, Porter S.

    Journal of Dental Research, 2005; 84(3):199-08.

30. Amelogenesis imperfecta with renal disease- a case report of two cases.

    Elizabeth J, Lakshmipriya E, Umadevi KMR, Ranganathan K.

    Journal of Oral Pathology and Medicine 2007; 16: 625-28.

31. Polycystin-1 Surface Localization Is Stimulated by Polycystin-2 and Cleavage at the G- Protein-coupledReceptorProteolytic Site.

    Chapin H. C. RajendranV,Caplan MJ.

Molecular Biology of the Cell 2010; 21: 4338-48,

32. Polycystin-1 Interacts with Intermediate Filaments.

    Xu GM, Sikaneta T, Sullivan BM, Zhang Q, Andreucci M, Stehle T, Drummond I, Arnaout MA.

    The Journal of Biological Chemistry 2001; 276: 46544–52.

33. Characterization of PKD protein-positive -like vesicle.

    Hogan MC, Manganelli L, Woollard JR, Masyuk AI, Masyuk TV,

    Journal of American Society of Nephrology 2009;20: 278–88.

34. Molecular pathogenesis of ADPKD: The polycystin complex gets complex.

    Ong ACM, Harris PC.

    Kidney International 2005; 67: 1234-47

35. The C-terminal tail of the polycystin-1 protein interacts with the Na,K-ATPase subunit.

    Zatti A, Chauvet V, Rajendran V, Kimura T, Pagel P, Caplan MJ.

    Molecular Biology of the Cell 2005; 16: 5087-93.

36. Characterization and cell distribution of polycystin, the product of autosomal dominant polycystic kidney disease gene1.

    Palsson. R, Sharma CP, Kim K, McLaughlin M, Brown M, Arnaout MA.

    Molecular Medicine 1996; 2: 702-11.

37. Polycystin-1 expression in fetal, adult and autosomal dominant polycystic kidney.

    Chae SW, Cho EY, Park MS, Lee KB, Kim H, Chae UKW, Cho EY, Kim H, Kim U.

    Journal of Korean Medical Science 2006; 21: 425-29.

38. Cellular Localization and Tissue Distribution of Polycystin-1.

Peters DJM, Wal AV, Sprut L, Saris JJ, Breuning MH, Bruijn JA, Heer ED.

Journal of Pathology 1999; 188: 439–46.

39. Functional polycystin-1 expression is developmentally regulated during epithelial morphogenesis in vitro: downregulation and loss of membrane localization during cystogenesis.

Bukanov NO, Husson H, Dackowski WR, Lawrence BD, Clow PA, Roberts BL, Klinger KW, Beskrovnaya OI.

Human Molecular Genetics 2002; 11: 923-36.

40. Analysis of the polycystins in aortic smooth muscle cells.

Qian Q, Li M, Cai Y, Ward CJ, Somlo S, Harris PC, Torres VE.

Journal of American Society of Nephrology 2003;14: 2280-87.

41. Stable knockdown of polycystin-1 confers integrin α2β1- mediated anoikis resistance.

Battini L, Fedorova E, Macip S, Li X, Wilson PD, Gusella GL.

Journal of American Society of Nephrology 2006; 17: 3049-58.

42. Structure - function relationships of the extracellular domain of the autosomal dominant polycystic kidney disease-associated protein, polycystin-1.

Weston BS, Malhas AN, Price RG.

Federation of European Biochemical Societies (FEBS) Letters 2003; 538:8-13.

43. The polycystic kidney disease proteins, polycystin-1, polycystin-2, polaris, and cystin, are co-localized in renal cilia.

Yoder BK, HouX,Woodford LMG.

Journal of American Society of Nephrology 2002; 13: 2508-16.

44. Polycystin-1, the product pf polycystic kidney disease 1 gene, co-localizes with desmosomes in MDCK cells.

Scheffers MS, van der Bent P, Prins F, Spruit L, Breuning MH, Litvinov SV, Heer ED, Peters JM.

Human Molecular Genetics 2000; 9 : 2743-50.

45. The cell biology of Polycystic kidney Disease

Chapin HC, Caplan MJ

Journal of Cell Biology 2010; 191: 701-10.

46. Mechanisms of disease: autosomal dominant and recessive polycystic kidney diseases.

Torres VE, Harris PC.

Nature clinical practice 2006; 2: 40-56.

47. Comparison of Pkd1 targeted mutants reveals that loss of polycystin-1 causes cystogenesis and bone defects.

Lu W, Shen X, Pavlova A, Lakkis M, Ward CJ. Pritchard L.

Human Molecular Genetics 2001;10: 2385-96.

48. Functional polycystin-1 dosage governs autosomal dominant polycystic kidney disease severity.

Hopp K, Ward CJ, Hommerding CJ, Nasr SH, Tuan HF, Gainullin VG.

Journal of Clinical Investigation 2012; 122:4257-73.

49. Multifocal renal cell carcinoma of different histological subtypes in autosominal dominant polycystic kidney disease.

Na KY, Kim HS, Park YK, Chang SG.

The Korean Journal of Pathology 2012; 46: 382-86.

50. Ciliary function of polycystin: a new model for cystogenesis.

Joly D, Hummel A, Ruello A, Knebelmann B.

Nephrology, Dialysis and Transplant Association 2003; 18: 1689-92.

51. Dental follicular tissue: misinterpretation as odontogenic tumors.

Kim J, Ellis G

Journal of Oral and Maxillofacial Surgery 1993; 51: 762-67.

52. Pathosis associated with radiographically normal follicular tissues in third molar impactions: A clinicopathological study.

Mesgarzadeh AH, Esmailzadeh H, Abdolrahimi M, Shahamfar M.

Indian Journal of Dental Research 2008; 19: 208-12.

53. Identification and localization of polycystin, the PKD1 Gene product.

Geng L, Segal Y, Peissel B, Deng N, Pei Y, Carone F, Rennka HG, Zhou J.

Journal of Clinical Investigations 1996; 98: 2674-82.

54. Loss of polycystin-1 in human cyst lining epithelia leads to ciliary dysfunction.

Nauli S, Rossetti S, Kolb RJ, Alenghat FJ, Consugar MB, Harris PC, Ingber DE, Zhou J.

Journal of American Society of Nephrology 2006; 17: 1015-25.

ANNEXURE -I

26/09/2011

From,

Institutional Review Board,

Ragas Dental College and Hospital,

Uthandi, Chennai

The dissertation topic titled **'Evaluation of Polycystin-1 Expression in Dentigerous Cyst and Asymptomatic Dental Follicle'** submitted by Dr.M.S.JaishLal has been approved by the Institutional Review Board of Ragas Dental College and Hospital on 26th September 2011.

Dr.K.Ranganathan
Secretary,
Ragas, IRB

Dr.S.Ramachandran
Chairman,
Ragas, IRB

ANNEXURE-II

DISSERTATION PROTOCOL

1. **Title of dissertation:**

   'Evaluation of Polycystin-1 Expression in Dentigerous Cyst and Asymptomatic Dental Follicle'

2. **Name and designation of the principal investigator:**

   Dr. Jaish Lal M.S.

   II Year Postgraduate student

   Department of Oral and Maxillofacial Pathology

3. **Name of HOD and staff incharge:**

   Dr. K. Ranganathan, M.D.S, M.S(OHIO) PhD.

   Dr. K. Umadevi, MDS

   Dr. Elizabeth Joshua, MDS

   Dr. T. Rooban, MDS

4. **Department where the project is to be carried out:**

   Study will be conducted in Department of Oral & Maxillofacial Pathology,

   Ragas Dental College & Hospital

5. **Duration of the project:**

   1 year

6. **Rationale:**

   Mutation of a calcium channel protein, Polycystin is being postulated to play a vital role in pathogenesis of dentigerous cyst. Polycystin mutation has been established as a major factor in pathogenesis of several cysts including odontogenic cysts. The role of this calcium channel interacting protein needs to be studied in pathogeneisis of dentigerous cyst.

7. **Hypothesis:**

- **Altered polycystin expression is observed in Dentigerous cyst.**

8. **Aim:**

1. To identify the light microscopic features of asymptomatic dental follicular tissue.
2. To study the expression of Polycystin-1 in dental follicular tissue.
3. To study the expression of Polycystin-1 in dentigerous cyst.
4. To compare 3 and 4.
5. To identify the prevalence of oral lesions in chronic renal disease patients.

9. **Objectives:**

To identify Polycystin in Dental follicle and Dentigerous cyst.

10. **Materials and methods:**

Sample: 40 cases of Dental follicles.

15 cases of Dentigerous cyst

100 cases of oral screening for Chronic Renal Disease

Procedure: Immunohistochemistry

Statistics to be used:

- The proportion between the groups is analyzed using Chi-Square analysis.
- Data analysis to be done using SPSS (Statistical Package Of Social Science) version 17.0

Equipments and chemical reagents needed:

Microtome	Slide warmer
Autoclave	Coupling jars
Hot air oven	Measuring jar

Weighing machine	Electronic timer
APES coated slides	Beakers
Slide carrier	Rectangular steel tray with glass rods
Aluminium foil	Sterile gauze
Micro-pipettes	Cover-slips
Toothed forceps	Light microscope

REAGENTS USED

1. Conc. HCl	7. 3% H2O2
2. Laxbro soln	8. Deionized distilled water
3. APES (3 amino propyl tri ethoxy silane)	9. Nuclear Fast Red
4. Acetone	10. Absolute alcohol
5. Citrate buffer	11. Xylene
6. Phospho Buffer Saline (PBS)	

ANTIBODIES USED

1. Primary antibody – Polycystin-1

2. Secondary antibody – ImmunoCruz™ mouse ABC Staining System

Detailed budget plan:

Antibodies – 36000Rs

Total – 40000Rs

Signature of principal investigator

Signature of Head of Department

Remarks of committee:

Permission granted **YES** **NO**

Modifications / condition

Annexure -III

S.No	Biopsy No	Age	Sex M/F	site of the dental follicle taken	Clinical features of the site			Histopathological features																						Others			
								Inflammation				Lining Epithelium								Odontogenic Rest		Calcification			Connective Tissue					Giant cells		Foreign bodies	
					Duration	Inflammation	Radiolucency	Acute	Chronic	mixed	diffuse	Present	absent	ParaKer	Orthokert	Non-keratinized	Acanthosis	Hyperplasia	Other Features	Present	Absent	Amorphous	Eosinophillic	Bone	Fibrous	Myxoid	Oedmatous	loose	Dense	Present	Absent	Present	Absent
1	4318/11	32	F	38	2 months	persent	radiolucency involving distal surface of tooth	nil	nil	mixed	nil	nil	nil	nil	nil	Non-Ker squamous	nil	nil	nil	nil	nil	nil	eosinophillic	nil	fibrous	nil	nil	nil	nil	present	nil	nil	nil
2	4320/11	22	M	43	1 month	persent	radiolucency involving distal surface of tooth	nil	nil	nil	nil	nil	nil	nil	nil	non-keratinised	nil	nil	Extravasated RBCs present	nil	nil	nil	eosinophilic	nil	fibrous	nil	nil	nil	nil	nil	nil	nil	nil
3	4435/11	20	F	28	6 months	persent	radiolucency involving coronal portion of tooth	nil	nil	nil	nil	nil	nil	nil	nil	non-keratinised	nil	nil	Extravasated RBCs present	Present	nil	nil	nil	vital bone	fibrous	nil	nil	nil	nil	nil	nil	nil	nil
4	4452/11	22	F	38	2months	persent	radiolucency involving coronal portion of tooth	nil	nil	mixed	nil	nil	nil	S.paraker	nil	nil	nil	nil	RBCs and nerve fibres present	nil	nil	nil	nil	vital bone	fibrous	nil	nil	nil	nil	nil	nil	nil	nil
5	4467/11	22	F	48	4 months	persent	radiolucency involving coronal portion of tooth	nil	nil	mixed	nil	nil	nil	nil	nil	non-ker	nil	nil	Extravasated RBCs present	nil	nil	nil	nil	nil	fibrous	nil	nil	nil	nil	nil	nil	nil	
6	4537/11	42	F	28	12 months	absent	radiolucency involving coronal portion of tooth	nil	nil	mixed	nil	nil	nil	nil	nil	non-keratinised	nil	nil	nil	present	nil	nil	nil	vital bone	fibrous	nil	nil	nil	nil	nil	nil	nil	nil
7	4537/11	42	F	38	12 months	absent	radiolucency involving coronal portion of tooth	nil	nil	mixed	nil	nil	nil	nil	nil	non-keratinised	nil	nil	nil	Present	nil	nil	nil	vital bone	nil	nil	nil	nil	nil	nil	nil	nil	nil
8	4552/12	28	F	48	2 months	persent	radiolucency involving distal surface of tooth	nil	nil	mixed	nil	nil	nil	nil	nil	non-ker	nil	nil	nil	nil	nil	nil	nil	nil	fibrous	nil	nil	nil	nil	nil	nil	nil	nil
9	4435/11	16	F	38	5 months	persent	radiolucency involving coronal portion of tooth	nil	nil	nil	nil	nil	nil	nil	nil	non-keratinised	nil	nil	nil	nil	nil	nil	focal areas of calcification		fibrous	nil	inflammed	nil	nil	nil	nil	nil	nil
10	4565/12	18	M	48	7 months	persent	radiolucency involving coronal portion of tooth	nil	nil	nil	nil	nil	nil	nil	nil	non-keratinised	nil	nil	nil	nil	nil	nil	calcification	nil	nil	nil	oedematous	nil	nil	nil	nil	nil	nil
11	4572/12	34	F	38	1 month	absent	radiolucency involving coronal portion of tooth	nil	chronic	nil	diffuse	nil	nil	nil	nil	non-ker	nil	nil	Extravasated RBCs present	present	nil	nil	basophillic	nil	fibrous	nil	nil	nil	nil	nil	nil	nil	nil
12	4573/12	48	F	48	2 months	absent	radiolucency involving coronal portion of tooth	nil	nil	nil	nil	nil	nil	nil	nil	non-keratinised	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil
13	4575/12	21	F	18	4 months	persent	radiolucency involving coronal portion of tooth	nil	nil	nil	nil	nil	nil	nil	nil	non-keratinised	nil	nil	nil	present	nil	nil	focal areas of calcification		fibrous	nil	nil	nil	nil	nil	nil	nil	nil
14	4587/12	21	M	48	2 months	absent	radiolucency involving coronal portion of tooth	nil	nil	nil	diffuse	nil	nil	nil	nil	nonk-keratinised	nil	nil	Exrtravasated RBCs	nil	nil	nil	nil	nil	fibrous	nil	inflammed	nil	nil	present	nil	nil	nil
15	4590/12	37	M	18	6 months	persent	radiolucency involving coronal portion of tooth	nil	nil	nil	nil	cystic lining present	nil	nil	nil	non-ker SSE	nil	nil	nil	nil	nil	nil	nil	nil	fibrous	nil	inflammed	nil	nil	nil	nil	nil	nil
16	4602/12	22	F	48	2 months	absent	radiolucency involving coronal portion of tooth	nil	chronic	nil	diffuse	nil	absent	nil	nil	non-keratinised	nil	nil	nil	nil	nil	nil	nil	vital bone	fibrous	nil	nil	nil	nil	nil	nil	nil	nil
17	4611/12	18	F	28	5 months	absent	radiolucency involving coronal portion of tooth	nil	chronic	nil	dense	nil	nil	nil	nil	non-ker SSE	nil	nil	nil	nil	nil	nil	basophilic	nil	fibrous	nil	inflammed	nil	nil	nil	nil	nil	nil
18	4613/12	19	F	38	1 month	absent	radiolucency involving distal surface of tooth	nil	nil	nil	nil	nil	nil	nil	nil	nonkeratintsed	nil	nil	Nerve Fibers	nil	nil	nil	nil	nil	fibrous	nil	nil	nil	nil	nil	nil	nil	nil
19	4622/12	21	F	18	1 month	absent	radiolucency involving distal surface of tooth	nil	nil	nil	nil	nil	nil	ciliated SSE	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	fibrous	nil	nil	nil	nil	nil	nil	nil	nil
20	4623/12	29	F	38	3 months	absent	radiolucency involving coronal portion of tooth	nil	nil	mixed	diffuse	nil	nil	nil	nil	non-kerSSE	nil	nil	nil	nil	nil	nil	nil	nil	fibrous	nil	nil	nil	nil	nil	nil	nil	nil
21	4625/12	28	F	48	4 months	persent	radiolucency involving coronal portion of tooth	nil	nil	mixed	diffuse	nil	nil	nil	nil	non-kerSSE	nil	nil	nil	nil	nil	nil	nil	nil	fibrous	nil	inflammed	nil	nil	nil	nil	nil	nil
22	4626/12	24	M	48	3 months	absent	radiolucency involving distal surface of tooth	nil	nil	nil	nil	nil	nil	para-ker SSE	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	fibrous	nil	inflammed	nil	nil	nil	nil	nil	nil
23	4651/12	18	F	48	2 months	absent	radiolucency involving coronal portion of tooth	nil	chronic	nil	nil	nil	nil	nil	nil	non-keratinised	nil	nil	Spicules of vital bone	nil	nil	nil	eosinophilic	vital bone	fibrous	nil	nil	nil	nil	nil	nil	nil	nil
24	4652/12	20	F	48	2 months	absent	radiolucency involving coronal portion of tooth	nil	nil	nil	nil	nil	nil	para-ker SSE	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	fibrous	nil	nil	nil	nil	nil	nil	nil	nil
25	4654/12	30	M	48	1 month	persent	radiolucency involving coronal portion of tooth	nil	chronic	nil	nil	nil	nil	squamous	nil	nil	nil	nil	Spicules of vital bone	nil	nil	nil	eosinophilic	vital bone	fibrous	nil	inflammed	nil	nil	nil	nil	nil	nil
26	4657/12	20	M	38	1 month	absent	radiolucency involving coronal portion of tooth	nil	chronic	nil	nil	nil	nil	nil	nil	non-keratinised	nil	nil	nil	nil	nil	nil	nil	nil	fibrous	nil	nil	nil	nil	nil	nil	nil	nil
27	4659/12	23	F	48	2 months	persent	radiolucency involving coronal portion of tooth	nil	chronic	nil	nil	nil	nil	para-ker SSE	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	fibrous	nil	inflammed	nil	nil	nil	nil	nil	nil
28	4666/12	21	M	48	2 months	persent	radiolucency involving distal surface of tooth	nil	nil	nil	nil	nil	nil	para-ker SSE	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	fibrous	nil	inflammed	nil	nil	nil	nil	nil	nil
29	4667/12	28	F	28	1 month	persent	radiolucency involving coronal portion of tooth	nil	chronic	nil	nil	nil	nil	para-ker SSE	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	fibrous	nil	inflammed	nil	nil	nil	nil	nil	nil
30	4678/12	17	F	38	4 months	persent	radiolucency involving coronal portion of tooth	nil	chronic	nil	nil	nil	nil	nil	nil	non-keratinised	nil	nil	nil	nil	nil	nil	nil	nil	fibrous	nil	inflammed	nil	nil	nil	nil	nil	nil
31	4690/12	29	M	48	2 months	absent	radiolucency involving distal surface of tooth	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	nil	Columnar Epithelium	nil	nil	nil	nil	nil	fibrous	nil	inflammed	nil	nil	nil	nil	nil	nil

ANNEXURE-IV

Chennai,
19/07/2012

From,

Dr. M.S. Jaish Lal
III year MDS
Department of Oral and Maxillofacial Pathology
Ragas Dental College and Hospital
Chennai.

Through,

Dr. K. Ranganathan
Professor and Head of the Department
Department of Oral and Maxillofacial Pathology
Ragas Dental College and Hospital
Chennai.

To,

The Managing Directors
Madurai Kidney Centre
Madurai.

Respected sir,

My thesis is on the Evaluation of polycystin-1 expression in dental follicles and dentigerous cyst, for a period of 1 year, which requires oral examination to be done on 100 patients with kidney disorders. The final thesis copy will be submitted to your esteemed kidney centre.

Thanking you.

Yours sincerely,
Dr. M.S. Jaish Lal

ANNEXURE -V

CASE SHEET

S. No:

Name: **Age:** **Gender:** Male/Female

Address:

Occupation: **Religion:** Hindu/Muslim/Christian/Others

Education: High school/College/Graduation

Dietary habits: Vegetarian / Mixed / Non-Vegetarian

ORAL HYGIENE:

Cleaning Material	Frequency / day	Method of Use	
		Brush	Finger
Tooth Paste / Powder / Others	Once / Twice / Thrice		

Medical History:

Diabetic: Hypertensive:

(Drugs) (Drugs)

Others:

NEPHROLOGY HISTORY:

Renal Stone (Duration)	Yes / No	If yes, since how Long?
Dialysis (Duration)	Yes / No	If yes, when?
Renal Failure (Duration)	Yes / No	(Acute / Chronic)
Renal Transplantation	Yes / No	If yes, when?
Polycystic kidney Disease (Familial history, duration, treatment)	Yes/ No	
Others		

Renal Drugs:

Oral Findings:

1.	Gingiva	NAD/ Lesion
2.	Buccal Mucosa	NAD/ Lesion
3.	Labial Mucosa	NAD/ Lesion
4.	Vestibule-Buccal	NAD /Lesion
	Labial	NAD /Lesion
5.	Tongue: Dorsal surface	NAD /Lesion
	Ventral surface	NAD /Lesion
	Lateral surface	NAD /Lesion
6.	Floor of the mouth	NAD /Lesion
7.	Angle of the mouth	NAD /Lesion
8.	Palate- Hard Palate	NAD /Lesion
	Soft Palate	NAD/ Lesion
9.	Uvula	NAD /Lesion
10.	Tonsilar area	NAD /Lesion
11.	Oropharynx	NAD /Lesion

Hard Tissue Finding:

No. of Teeth present:

No. of Teeth absent:

No. of Teeth Decayed:

No. of Teeth Filled:

Laboratory Findings:

Description of oral findings:

Provisional Diagnosis:

ANNEXURE –VI

ACUTE RENAL FAILURE	CHRONIC RENAL FAILURE
Acute renal failure (ARF) is a syndrome characterized by rapid decline in glomerular filtration rate (hours to days), retention of nitrogenous waste products, and perturbation of extracellular fluid volume and electrolyte and acid-base homeostasis.	**Chronic renal disease (CRD)** is a pathophysiologic process with multiple etiologies, resulting in the inexorable attrition of nephron number and function and frequently leading to **end-stage renaldisease (ESRD).**
Results in retention of toxins, fluids, and end products of metabolism	Results from gradual, progressive loss of renal function. Occasionally results from rapid progression of acute renal failure
Laboratory Manifestations Of Acute Renal Failure ARF is usually asymptomatic and diagnosed when biochemical monitoring of **increase** in blood **urea** and **creatinine** concentrations.	**Laboratory Manifestations Of Chronic Renal Failure** ➢ Increased in **Albumin, Urea, Creatinine**, **Potassium, Sodium and Water Homeostasis** ➢ Decreased **Ammonia** and increased **acidosis** ➢ Decreased **Calcium** and **Phosphate**
Usually reversible with medical treatment	**ESRD** represents a clinical state or condition in which there has been an irreversible loss of endogenous renal function, of a degree sufficient to render the patient permanently dependent upon renal replacement therapy (dialysis or transplantation) in order to avoid life-threatening *uremia*. Diabetic and hypertensive nephropathy are the leading underlying etiologies of both CRD and ESRD.

ORAL LESIONS:

CANDIDIASIS:

White or yellow spots or plaques that may be located in any part of the oral cavity and can be wiped off to reveal an erythematous surface which may bleed.

ANGULAR CHELITIS:

It is a chronic multifocal candidiasis involves the angle of the mouth, characterized by erythema, fissuring and scaling.

PIGMENTATION:

Oral pigmentation may be physiological or pathological in nature. It may represent a localized anomaly of limited significance or the presentation of potentially life-threatening multisystem disease. A full history of evolution of the pigmentary changes, as well as inquiring into family history, drug ingestion and systemic symptoms of concurrent disease are clearly important in the assessment.

TRAUMATIC ULCER:

A sharp edge of a tooth, accidental biting (this can be particularly common with sharp canine teeth, or Wisdom teeth), sharp, abrasive, or excessively salty food, hot drinks, poorly fitting dentures, dental braces or trauma from a toothbrush may injure the mucosal lining of the mouth resulting in an ulcer.

UREMIC STOMATITIS:

It represents a relatively uncommon complication of renal failure Nevertheless, for the patients in whom uremic stomatitis develops, this can be a painful disorder. The cause of the oral lesions is unclear, but some investigators suggest that urease, an enzyme produced by the oral micro flora, may degrade ureasecreted in the saliva. This degradation results in the liberation of free ammonia, which presumably damages the oral mucosa.

DENTAL CARIES:

Fissures, pits and grooves occurs on the occlusal sutface with black pigmentation, catch on probing cavitation. It is a chronic disease that leads to pain and disability across all the age groups.

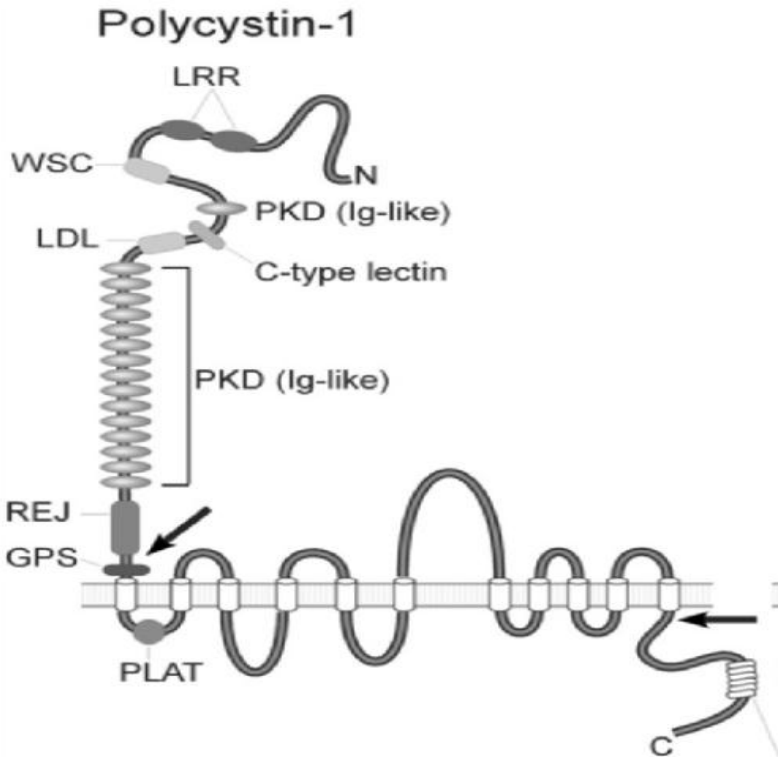
CHRONIC GINGIVITIS:

Inflammation of gingiva is termed as gingivitis. Bleeding on probing is the clinical finding.

CHRONIC PERIODONTITIS:

Periodontitis refers to an inflammation of the gingival tissues in association with some loss of both the attachment of the periodontal ligament and bony support. Periodontitis characterized by soft tissue loss as a result of ulceration or necrosis. Exposure, destruction or sequestration of bone may be seen, and the teeth may become loosened. Pain may be a prominent feature.

ANNEXURE-VII



(Fig:1) The structure of polycystin- 1 (LRR, leucine-rich repeats; WSC, cell wall integrity and stress response component 1; PKD (Ig-like), Ig-like domains; LDL, low density lipoprotein domain; REJ, receptor for egg jelly; GPS, proteolytic G protein coupled receptor proteolytic site; PLAT, lipoxygenase domain.[11]

In a study by **Peters DJM et al,**[38] using several polyclonal antibodies for specific regions of Polycystin-1, in several human tissues and Madin Darby canine kidney (MDCK) cell lines identified the following features.

1. In Kidney: Epithelia of Bowman's capsule, proximal tubules, distal tubules, and collecting ducts were polycystin-1positive with variable intensities.
2. Visceral epithelium was positive on cryostat sections, but virtually negative in archival paraffin sections.
3. Adrenal gland show positive staining in compact cells (glucocorticoid-producing cells) in the zonafasciculata of the cortex; zonaglomerulosa and zonareticularis negative while medulla was not included.
4. Liver: Hepatocytes were positive; epithelium lining small intra-hepatic biliary ductules and large bile ducts positive, while other structures in portal tract negative.
5. Pancreas: Exocrine component: epithelial cells of the intercalated, inter- and intralobular ducts positive, acini very weak to negative. Endocrine component: intense staining in the endocrine cells in the pancreatic islets, most intense in the insulin-producing cells; fine collagenous network with capillaries and capsule negative.
6. Spleen: Most structures and cells do not stain, except a few unidentified cells.
7. Colon: Epithelium of the mucosa positive: the luminal surface (absorptive cells) more intense than the crypts (predominantly mucus-secreting cells). Sometimes an unidentified cell in the lamina propria, probably plasma cell, intensely positive.
8. Tonsils: Negative.
9. Thyroid: Negative.
10. Parathyroid: Polycystin-1 staining in parathyroid hormone-producing cells. The oxyphil cells and septa are negative.

11. Lung: The columnar ciliated epithelial cells in the trachea are positive but the goblet cells scattered among them do not stain. Lamina propria shows non-specific staining.
12. Prostate gland: Epithelial cells of a subset of ducts in the main prostatic gland stain positive. Lamina propria negative.
13. Testis: Seminiferous tubules with spermatocytes show non-specific staining. The testosterone-producing Leydig cells located in the interstitial supporting tissue are strongly positive but other interstitial cells are negative.
14. Endometrium Columnar and cuboidal epithelium of tubular glands positive. Stroma negative.
15. Pituitary gland: In adenohypophysis, the chromophilic and chromophobic secretory cells are located in clusters surrounded by a basement membrane. Chromophilic cells are postive while the chromophobic cells are negative.Posterior pituitary negative.
16. Synovium: Negative
17. Skeletal muscle: Weak staining throughout the cytoplasm in myofibres. Perimysium with capillaries negative.
18. Heart muscle and cardiac valves: Smooth muscle cells in the lamina fibrosa of the tricuspid, bicuspid, pulmonic, and aortic valves are weakly positive, which remained so after trypsin treatment; endothelium negative. Myocytes of the striated heart muscle are clearly positive throughout the fibre but negative after trypsin treatment.

19. Brain vessels: Myocytes in tunica media and tunica intima are weakly positive without trypsin treatment and more intensely positive after trypsin treatment. Elasticainterna and tunica adventitia negative.